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Yield and Zinc Content of Sugar Beets as Affected by Nitrogen Source, Rate of Nitrogen, and Zinc Application[1]

LOUIS C. BOAWN, FRANK G. VIETS, JR., C. E. NELSON, AND C. L. CRAWFORD[2]

Received for publication March 14, 1960

Introduction

An experiment in which field plots were treated with a factorial combination of three nitrogen sources, three rates of nitrogen, and five rates of zinc was started at Prosser, Washington, in 1953. The cropping sequence on these plots included grain sorghum in 1953, potatoes in 1954, and sugar beets in 1955. The interest in sugar beets was concerned primarily with the influence of the treatments on zinc nutrition. At the same time, however, it was convenient to observe the effect of nitrogen rate and nitrogen source on such things as yield, sugar content, and petiole nitrate.

Apparently little work has been done to determine the zinc requirements of sugar beets. In a previous paper Boawn and Viets (1)[3] published data for a number of experiments showing levels of zinc in leaf blades, and concluded that 10 ppm. zinc in young, fully developed leaves at mid-season is adequate for above average yields. They did not report zinc levels in other parts of the plant nor the total zinc requirement. Lingle and Holmberg (5) have reported increased growth of sugar beets in Yolo County of California from applications of zinc but did not show levels of zinc in the plant associated with this response.

In the present experiment it was hoped that unfertilized plants would be sufficiently low in zinc that a growth response to additions of zinc would be obtained. This did not occur, and therefore the data do not establish "critical" levels for zinc in the tissues analyzed. On the other hand, since additional zinc did not increase growth, the levels of zinc found in plants from plots which did not receive zinc fertilization are shown to be adequate.

Procedure

Complete details of the experiment—procedure, objectives, and complete experimental results—have been given in two previ-

[1] Contributions from Soil and Water Conservation Research Division, Agricultural Research Service, U. S. Department of Agriculture, Washington Agricultural Experiment Station cooperating.

[2] Soil Scientist, Soil Scientist, formerly Soil Scientist, and Senior Scientific Aide, respectively, Western Soil and Water Management Research Branch.

[3] Numbers in parentheses refer to literature cited.

ous publications (2) (3). Only that portion of the procedure pertinent to the growing of sugar beets will be repeated here.

The soil at the experimental site was a Ritzville fine sandy loam, a Brown soil which is noncalcereous to a depth of 18 inches and is essentially neutral at the surface. Total nitrogen is low, about 0.04 percent in the surface, and decreases gradually with depth. The area had not been cropped previously and was taken out of sagebrush and bunchgrass just prior to application of the treatments.

Treatments consisted of three nitrogen carriers, three rates of nitrogen, and five rates of zinc applied in factorial combination, Nitrogen carriers were $(NH_4)_2SO_4$, NH_4NO_3, and $Ca(NO_3)_2$; nitrogen rates 40, 80, and 160 pounds N per acre; and Zn rates 0, 2, 4, 8, and 16 pounds per acre as $ZnSO_4$. The treatments were replicated twice in a randomized block design on 20 x 33 foot plots. In addition to these treatments, data were taken from plots which received neither N nor Zn. These plots were located among the other plots but were not randomized.

$ZnSO_4$ was applied only once, at the initiation of the experiment in 1953. Sugar beets, which were grown on the plots in 1955, therefore measured effectiveness of zinc fertilizer after contact with the soil for two years. Nitrogen treatments were made annually at the initial rates. All materials were broadcast on the surface and rototilled to a depth of eight inches the first year. In subsequent years the nitrogen materials were broadcast and plowed down.

The beets were furrow-irrigated and all cultural operations were done according to standard grower practice.

Leaf blades for analysis were taken on August 24 and consisted of 20 young, fully-developed leaves without petioles from each plot. Total top samples were taken August 25 and 26 from two 10-foot sections of row from each plot. Yield data are based on hand-topped beets from two 10-foot sections of row from each plot. The harvest date was October 24. Subsamples of roots for chemical analysis were obtained from the harvested beets using a Spreckels saw.

Zinc was determined by the dithizonate procedure essentially according to Holmes (4) using nitric-perchloric digests. Samples for sugar analysis were taken according to the procedure of the Utah-Idaho Sugar Company and the analysis was made by them along with samples from farmers' beets. Sugar analyses are expressed on a fresh weight basis; zinc and nitrate analysis on an oven-dry basis.

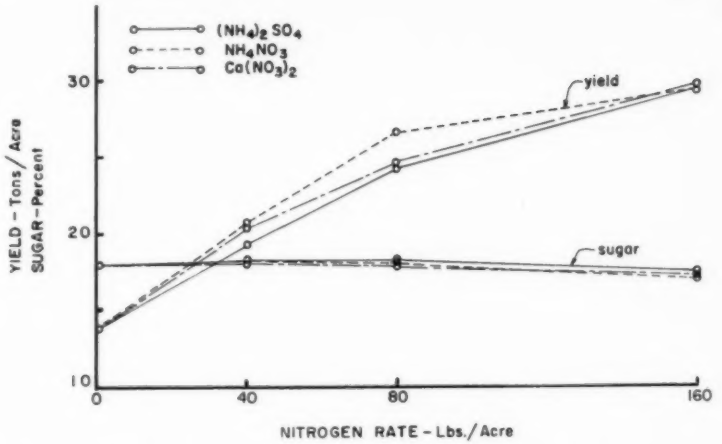

Figure 1.—Tared yield and sugar percentage of beets as affected by nitrogen rate and nitrogen carrier. (L.S.D. for nitrogen rate at the 1 percent level: yield—1.85 tons/acre, sugar—0.68 percent. Nitrogen carrier differences not significant at the 5 percent level.)

Results and Discussion

Yield and Sugar Percentage

Figure 1 shows the effect of nitrogen rate and carrier on beet yield and sugar percentage. Without nitrogen fertilization the yield of beets was slightly more than 14 tons per acre and increased sharply with each rate of nitrogen to just slightly less than 30 tons when the N rate was 160 pounds per acre. As shown by Table 1 the petiole nitrate-nitrogen even in those plots receiving 160 pounds of nitrogen per acre had dropped to 210 ppm. nitrogen as early as August 24, far below Ulrich's critical concentration of 1000 ppm. (6). It is evident that higher yields could have been obtained had an adequate supply of nitrogen been available during the early part of September. There was a tendency for $Ca(NO_3)_2$ and NH_4NO_3 to result in higher yields than were obtained with $(NH_4)_2SO_4$ but the differences are not statistically significant at the 5 percent level.

Sugar percentage in harvested beets decreased from 18.13 percent to 17.11 percent as the rate of nitrogen fertilization was increased from 40 pounds per acre to 160 pounds per acre. This occurred even though the petiole nitrate-nitrogen had dropped to 210 ppm. as early as August 24 (Table 1), and the beets were not harvested until two months later on October 24.

Table 1.—Nitrate Nitrogen in Petioles at Three Sampling Dates (ppm. dry weight).

N Rate	Sampling Date		
	July 14	August 5	August 24
lbs./A.			
0	140	35	75
40	320	70	98
80	1530	96	58
160	6440	2120	210

Figure 2 shows mean beet yields (all rates of nitrogen) and mean sugar percentage in relation to levels of zinc fertilization. Considerable variation in yield is shown but was not found to be associated with zinc rate nor nitrogen carrier at the 5 percent probability level. The interaction between nitrogen carrier and zinc rate and between nitrogen rate and zinc rate were not significant either. Since the highest average yield of beets was obtained at the 0 level of zinc it can be concluded that these plants had adequate zinc to satisfy their growth requirements. Indeed, as shown by Figure 3, the leaf blades from these plants averaged about 20 ppm. zinc, which is much higher than the critical level of 10 ppm. previously reported (1).

Sugar percentage in harvested beets was unaffected by the level of zinc fertilization.

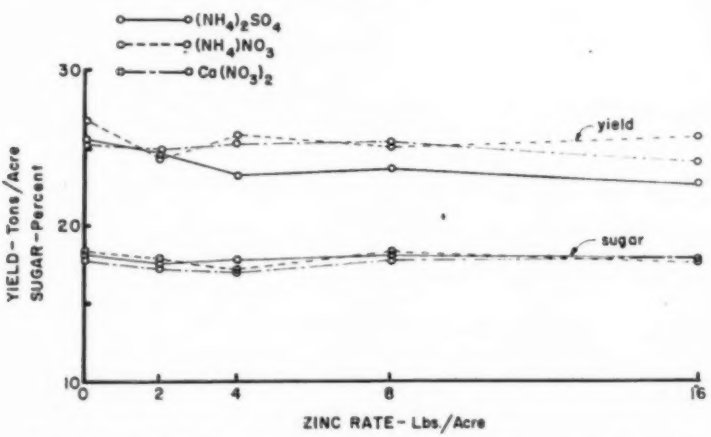

Figure 2.—Tared yield and sugar percentage of beets as affected by zinc rate and nitrogen carrier. Means of three nitrogen rates. (No significant differences at the 5 percent level.)

Zinc Concentration in Total Tops, Leaf Blades, and Roots

Figures 3 and 4 show the levels of zinc found in total tops, leaf blades, and roots as affected by different rates of zinc fertilization, nitrogen rate, and nitrogen carrier. Where 16 pounds zinc per acre was applied, the zinc concentration in leaf blades was increased by approximately 50 percent, from about 20 ppm. to a little over 30 ppm. In comparison, zinc concentration in total tops was increased from 12.5 ppm. to an average of 22 ppm., an increase of around 75 percent. Since zinc concentration in the total top is more responsive to the zinc status of the soil than leaf blades, further separation and analysis of the beet top would probably reveal a tissue which is more sensitive than either of these two.

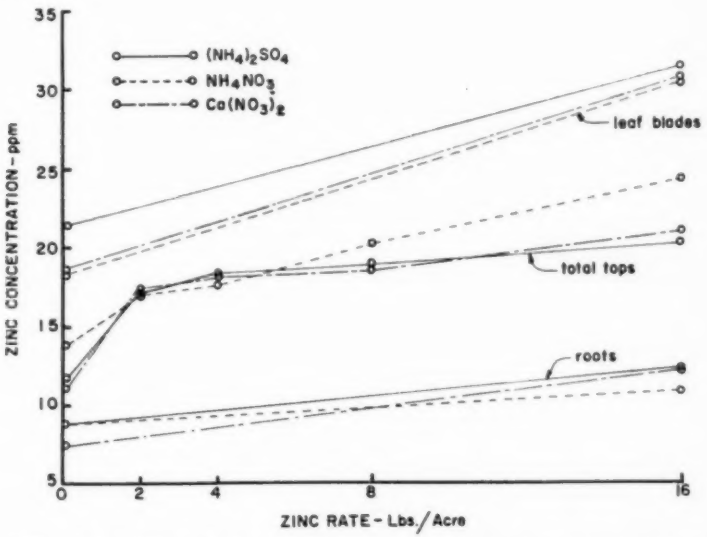

Figure 3.—Effect of zinc and nitrogen carrier on zinc concentration of total tops, leaf blades, and roots of sugar beets. Means of three nitrogen rates. (L.S.D. for zinc rate at the 1 percent level: total tops— 2.3 ppm., leaf blades—2.4 ppm., roots—1.6 ppm. Nitrogen carrier differences not significant at the 5 percent level.)

As was expected, the zinc uptake response to zinc rates was curvilinear, the major increase occurring with the first increment of 2 pounds per acre.

Zinc levels in beet roots were found to be much lower than in tops and to be less responsive to changes in zinc levels in the soil.

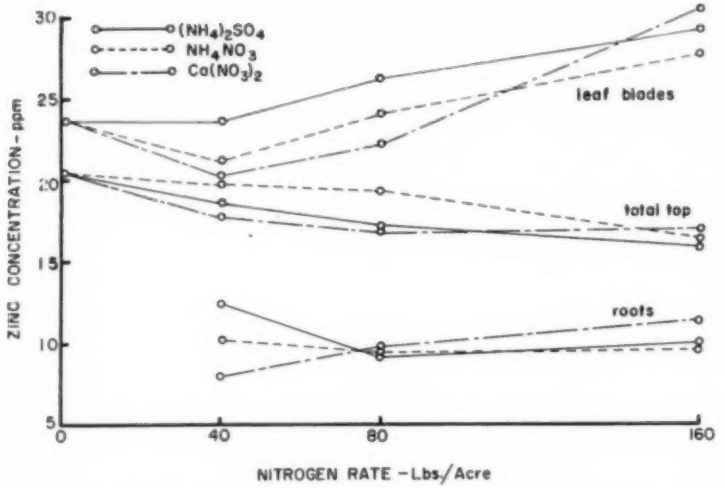

Figure 4.—Effect of nitrogen rate and nitrogen carrier on zinc concentration of total tops, leaf blades, and roots of sugar beets. Means of all zinc rates for total tops, and two zinc rates for leaf blades and roots. (L.S.D. for nitrogen rate at the 1 percent level: total top—1.8 ppm., leaf blades—3.0 ppm., roots—2.0 ppm. Nitrogen carrier differences not significant at the 5 percent level.)

Nitrogen carrier appears to have had some effect on the zinc concentration of the various plant parts, but in general it was not consistent and is not statistically significant at the 5 percent level.

Nitrogen fertilization increased the zinc concentration of leaf blades but decreased the zinc concentration of total tops. Apparently, as plant size was increased through nitrogen fertilization there was a substantial increase in the ratio of total top to leaf blades. That such a phenomenon does occur has been previously reported by Ulrich (7).

There would seem to be evidence that increasing rates of nitrogen actually increased the availability of zinc, as has been reported previously by the authors (2), but that in the case of total tops the increase in dry matter overshadowed the increased uptake of zinc.

Nitrogen rates did not change the concentration of zinc in roots.

The total zinc contained in sugar beet plants grown with two levels of zinc and three rates of nitrogen is given in Table 2.

Table 2.—Average Pounds Zinc Per Acre Contained in Sugar Beet Tops and Roots Grown with Three Rates of Ammonium Nitrate and Two Rates of Zinc.

Zn Rate	N Rate	Zinc Content		
		Tops	Roots	Sum
lbs./A.	lbs./A.		lbs./A.	
0	40	.044	.108	.152
	80	.063	.100	.163
	160	.069	.114	.183
16	40	.073	.108	.181
	80	.090	.142	.232
	160	.140	.128	.268

In comparison to other crops sugar beets remove fairly large amounts of zinc from the soil. This is especially true if tops as well as roots are removed. The amount of zinc removed by sugar beets may well be a factor in the common observation that corn planted on ground which has grown sugar beets continuously for several years will usually show severe zinc deficiency symptoms.

Summary and Conclusions

Sugar beets were grown the third year of a cropping sequence on plots treated with a factorial combination of three nitrogen carriers, three rates of nitrogen, and five rates of zinc. Increasing the nitrogen rate from 0 to 160 pounds per acre increased the beet yields from 14 tons per acre to about 30 tons per acre. Nitrogen carrier had no effect on yield. The rates of nitrogen applied caused the average sugar percentage to decrease from 18.13 percent to 17.11 percent. Zinc rates from 0 to 16 pounds per acre had no effect on either yield or sugar percentage.

Nitrogen rate and nitrogen carrier had no effect on the zinc content of various plant parts. Sixteen pounds zinc per acre, applied 2 years earlier, increased the zinc content of leaf blades from 20 ppm. to slightly over 30 ppm., of total tops from 12 ppm. to 22 ppm., and of roots from 8 ppm. to 12 ppm. These increases in the level of zinc in the plant did not produce a measurable increase in beet yield.

The total zinc contained in a beet crop yielding 30 tons per acre varied from 0.183 pounds per acre to 0.268 pounds per acre depending on the level of zinc fertilization.

Literature Cited

(1) BOAWN, LOUIS C., and VIETS, FRANK G., JR. 1956. Zinc fertilizer tests on sugar beets in Washington. Jour. Amer. Soc. Sugar Beet Tech. 9 (3): 212-216.

(2) BOAWN, LOUIS C., VIETS, FRANK G., JR., CRAWFORD, C. L. and NELSON, J. L. 1960. Effect of nitrogen carrier, nitrogen rate, zinc rate, and induced soil changes on zinc uptake by sorghum, potatoes, and sugar beets. Soil Sci. 90: 329-337.

(3) BOAWN, LOUIS C., NELSON, C. E., VIETS, FRANK G., JR., and CRAWFORD, C. L. 1960. Nitrogen carrier and nitrogen rate influence on soil properties and nutrient uptake by crops. Wash. State University., Agr. Exp. Sta. Bul. 614.

(4) HOLMES, R. S. 1945. Determination of total copper, zinc, cobalt, and lead in soils and soil solutions. Soil Sci. 59: 77-84.

(5) LINGLE, JOHN C., and HOLMBERG, D. M. 1956. Zinc-deficient crops. Calif. Agr. 10 (2): 13-14.

(6) ULRICH, A. 1950. Critical nitrate levels of sugar beets established from analysis of petioles and blades, with special reference to yields and sucrose concentrations. Soil Sci. 69: 291-309.

(7) ULRICH, A. 1955. Influence of night temperature and nitrogen nutrition on growth, sucrose accumulation, and leaf minerals of sugar beet plants. Plant Physio. 30: 250-257.

Movement and Persistence of Endothal (3,6-Endoxohexahydrophthalic Acid) as Influenced by Soil Texture, Temperature, and Moisture Levels[1]

R. D. Comes, D. W. Bohmont, and H. P. Alley[2]

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Introduction

Many chemicals have been screened for selective weed control in sugar beets with varying degrees of success. The herbicide 3,6-endoxohexahydrophthalic acid, hereafter referred to as endothal, has given excellent results in some trials as a pre-emergent treatment, while other trials have been highly disappointing. Success or failure appears to depend largely upon the method in which moisture is received after application of the herbicide. It is generally accepted that, to obtain satisfactory results, moisture in the form of rainfall or sprinkler irrigation is necessary within a short period of time after application of the chemical.

Since endothal is known to be more effectively absorbed by roots than by foliage, this study was conducted with the following purposes: 1—to study the movement pattern of endothal in soil, 2—to determine the breakdown rate of endothal, and 3—to investigate some of the possible factors causing variation in endothal movement and persistence in soil.

Procedure

Movement of Endothal

Preliminary experiments were conducted to study the vertical movement of endothal in three different soil textures without regard to soil temperature or moisture. Three Wyoming soils with the following characteristics were used in these studies:

Characteristics of Soils[1]

Soil Type	% Sand	% Silt	% Clay	Organic Matter Percent	pH
Hill sandy clay loam	62.4	14.8	22.8	1.89	7.50
Greeley fine sandy loam	68.4	19.8	11.8	1.26	7.35
Billings clay loam	36.0	35.2	28.8	2.24	7.50

[1] Analyses were made by the University of Wyoming Soil Testing Laboratory, Plant Science Division, Laramie, Wyoming.

[1] Published with approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 147.

[2] Graduate Research Assistant, Head of Plant Science Division, and Assistant Professor, respectively, College of Agriculture, University of Wyoming.

All soils were dried and screened through a 2-millimeter screen. Each of the three soils was placed in separate 6-inch pots and treated with endothal at the rate of 4 lbs. (acid equivalent) per acre on an area basis. The material was applied at the following depths: surface, ½ inch to 1½ inches and 1½ inches to 3 inches. Hereafter, all rates refer to acid equivalent per acre.

The vertical movement of the herbicide in the soil was determined by biological assay. Norland flax was employed as the test plant since in the germinating stage it is very susceptible to injury by endothal.

Ten flax seeds were planted at a depth of ½ inch in all treated soils. Untreated pots of each soil texture were planted in the same manner. After planting, three pots of each soil treatment were subirrigated and three were sprinkle irrigated. These methods of irrigation were continued throughout the course of the experiment.

Thirty days after the planting date, all plants were counted and harvested. Reduction in total green weight of the flax plants was used as a measure of the presence, and therefore the movement, of endothal.

Subsequent movement studies were made with the same three soils. The air-dry soils were placed in 3-inch galvanized metal tubes and packed to simulate the soil conditions of a well-prepared seedbed. Packed height of the soil columns was 8 inches. Half of the soil columns were then wetted to field capacity. The air-dry and the field-capacity soil columns were placed in temperature-control chambers at 10, 15, 20, 25, and 30° C. ± 1°. Twenty-four hours were allowed for the soil temperatures to reach an equilibrium with the control chambers before endothal was applied to the surface of each column at the rate of 4 lbs. per acre. Eight hours after chemical treatment, all soil columns were leached with 2 surface inches of water. Twenty-four hours after leaching, the soil columns were sliced, into five layers.

Each section in reference to the soil surface was as follows: 0 to 1 inch, 1 inch to 2 inches, 2 inches to 3 inches, 3 inches to 4 inches, 4 inches to 6 inches, and 6 inches to 8 inches. Each soil section was placed in a 3-inch waxed-paper container which allowed for subirrigation so that further leaching of the chemical would not be a factor.

Ten flax seeds were planted in each soil section and subirrigated in the greenhouse, periodically as needed. Untreated soil of each type was planted with flax at the same time and used for comparison with the various treatments. Thirty days after planting, green weight of flax plants was measured as before.

Persistence of Endothal

Studies were conducted to determine the effect of temperature and moisture levels on the rate of disappearance of endothal in different soil textures.

Soil, 1½ inches deep, was surface treated with endothal in an aqueous solution at a rate of 6 lbs. per acre. Application of the chemical was made under simulated field conditions with a knapsack and boom sprayer. The total volume of treated soil was then placed in a tumbler mixer and thoroughly mixed to obtain a uniform distribution of the chemical in the soil. Half of each soil treatment was wetted to 80 percent of field capacity and the remaining half left in an air-dry condition.

The soils were then placed in five-quart containers and covered with glass panes to allow aeration but to conserve moisture, and placed in the temperature-control chambers.

Triplicate 10.6-cubic-inch soil samples were removed from each chemical treatment and from the untreated control 7 days after the initiation of the experiment and every 7 days thereafter for 56 days. The soil samples were placed in waxed-paper containers, planted with 10 flax seeds, and subirrigated periodically.

Flax plants were harvested at the soil level 21 days after planting and the green weight taken as a criterion for the presence of endothal.

Experimental Results

Movement of Endothal

Endothal moved in the same general direction as the water in sandy loam and sandy clay loam soils. The chemical, applied on the surface of these soils at the rate of 4 lbs. per acre, and sprinkle irrigated, reduced green flax weight by 91 percent in the sandy clay loam soil, 93 percent in the sandy loam, and 77 percent in the clay loam, as compared with the untreated check. Movement of the chemical by subirrigation of this treatment reduced green flax weight by 31 percent on the sandy clay loam soil with no reduction occurring in green flax weight on the sandy loam soil. Endothal, when applied on the surface of clay loam soil and subirrigated, reduced the flax weight by 86 percent. Apparently, the chemical moved downward when the soil was saturated, which is a reverse from the observed movement in sandy-type soils.

Conversely, subirrigation moved the greatest amount of endothal when the chemical was incorporated into the soil at depths of ½ to 1½ and 1½ to 3 inches (Table 1). When the

Table 1.—Average Green Weight of Flax from 3 Soil Types, Treated with 4 Pounds of Endothal per Acre at 3 Soil Levels, Using Two Methods of Irrigation.

Method of Irrigation	Soil Type	Depth of Chemical from Surface in Inches	Grams Green Weight	Percent Reduction of Green Weight Based on Untreated Check
Sprinkle Irrigated	SCL[1]	0	.10	90.6
		½-1½	.20	81.1
		1½-3	1.07	0.0
	SL[2]	0	.07	92.8
		½-1½	.40	58.8
		1½-3	.67	30.0
	CL[3]	0	.13	77.2
		½-1½	.27	52.6
		1½-3	.60	0.0
Sub Irrigated	SCL	0	1.20	30.6
		½-1½	0	100.0
		1½-3	.23	86.7
	SL	0	1.73	0.0
		½-1½	.37	77.3
		1½-3	.43	73.6
	CL	0	.20	85.7
		½-1½	.15	90.7
		1½-3	.17	87.9

[1] Sandy Clay Loam Soil.
[2] Sandy Loam Soil.
[3] Clay Loam Soil.

chemical was placed at these 2 soil depths and subirrigated, chemical movement was similar for both depths in all soils observed.

Methods of irrigation were not as critical when the chemical was incorporated in the ½- to 1½-inch depth. However, subirrigation of the treated soils consistently resulted in the greater movement of endothal as compared with the sprinkler irrigation method.

Additional chemical-movement studies indicated that soil texture was very influential on the amount of endothal retained in the top 1 inch of soil when leached with 2 surface inches of water. The chemical was more resistant to leaching in clay loam and sandy clay loam than in sandy loam soil. The two finer textured soils retained a greater amount of the chemical in the surface two inches of soil than at any other depths tested (Figure 1).

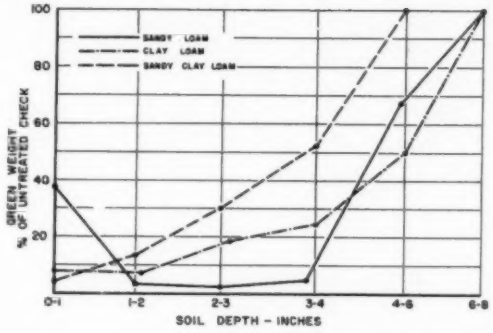

Figure 1.—Movement of endothal through three soil types after application of 2 surface inches of water, as measured by flax growth.

These results show that 87 percent more endothal was present 1 to 4 inches below the soil surface than on the soil surface of the sandy loam soil. There were no significant differences between the amount of chemical retained in the three soil layers within the 1- to 4-inch increment in the sandy loam soil.

Initial soil moisture did not have a significant effect on the mobility of endothal when leached with two surface inches of water.

Persistence of Endothal

The moisture content of the soil proved to be a very critical factor in the disappearance of endothal. Air-dry soils, which had been treated with 6 lbs. of endothal per acre and held in an air-dry condition for 8 weeks before the planting of flax, yielded no flax when irrigated. Results were the same for all temperatures employed.

Temperature also was very influential on the inactivation rate of endothal in soils that had been wetted to 80 percent of field capacity. There is a highly significant difference between the amount of chemical that remained in soils held at the 15° and 20° C. temperatures within 14 days after treatment. Inactivation, as measured by flax weight, was 12 times more rapid at 20° C. than 15° C. one week after treatment. There was no significant difference in the persistence of endothal among temperatures of 20, 25, and 30° C. nor between the two lower temperatures, 10 and 15° C.

The chemical disappeared more slowly in clay loam soil than in the sandy loam and the sandy clay loam soils which had been

wetted. Sufficient breakdown had occurred within 14 days after treatment in sandy-type soils, held at 20° C. and higher, to allow weed seeds to germinate and grow. Endothal in clay loam soil did not exhibit any measurable degree of inactivation until 28 days after treatment. Inactivation of the herbicide was completed 42 days after treatment in all soils and at all temperatures employed.

Discussion and Summary

Endothal is very mobile in the three soil textures studied. The chemical was transported by water movement, either in an upward or downward direction, depending upon the type of irrigation. In the field application of endothal it may be possible to side-dress sugar beets 1½ to 2 inches deep with the chemical and expect the inward and upward movement of water from furrow irrigation to carry the herbicide into the weed-germinating zone.

The amount of chemical retained in the top 1 inch of soil after leaching with 2 surface inches of water depended primarily upon soil texture. The chemical was leached more readily in sandy loam soil than in clay loam or sandy clay loam soil. These greenhouse trials suggest that excessive sprinkler irrigation or precipitation on sandy soil may leach the bulk of the chemical below the germinating zone of most annual weeds.

Dry soils apparently do not hold endothal with any greater force than soils which have been wetted to field capacity, once precipitation is received. The depth to which the herbicide will leach undoubtedly depends on the amount of precipitation received and the moisture content of the soil at that time.

Endothal became quite inactive seven days after treatment in the moist sandy-type soils which had been held at temperatures of 20° C. and higher. Endothal in clay loam soil did not show any degree of chemical inactivity until 28 days after treatment. It would seem that clay loam soil, having a larger surface area and containing a higher percentage of organic matter than either of the sandy soils, would inactivate endothal more readily.

Biological activity may play an important role in the inactivation of endothal for the following reasons: 1—no flax seed germinated in any soil type at any temperature, when the soil was in an air-dry condition. This was true even eight weeks after treatment. 2—Temperatures of 20° C. and higher increased the rate of inactivation in moist soils. Volatilization of the herbicide is apparently not a critical factor in dissipation, as the dry soils were free to aeration within the temperature chambers, and the chamber doors were opened every seven days for sampling.

Conclusions

1. It is possible to move endothal down or up in soil by using surface or subirrigation, respectively.
2. Endothal in sandy loam soils is more susceptible to leaching than in clay soils.
3. Endothal is leached to a depth of at least 3 inches in the 3 soil types studied, with 2 surface inches of water.
4. An application of 2 surface inches of water leached endothal as readily in an air-dry soil as in a soil wetted to field capacity.
5. The persistence of endothal was influenced by soil texture. The chemical was inactivated more rapidly in sandy loam and sandy clay loam than in clay loam soil.
6. Soil moisture plays a very important role in the dissipation of endothal. No inactivation of the chemical was measurable 8 weeks after treatment in air-dry soil.
7. The rate of inactivation of endothal was significantly less at temperatures below 20° C. than at temperatures of 20, 25, and 30° C.

Heterodera schachtii, Hatching Properties of Field Importance[1]

DAVID R. VIGLIERCHIO[2]

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Biological studies with the sugar beet nematode would be greatly facilitated by a continuing large supply of cysts of known origin and predictable character. For the development of large scale rearing of *Heterodera schachtii* it was of importance to have some knowledge of what events took place during the increase in population. In the course of experiments to elucidate the behavior of the plant parasites, knowledge was obtained which was useful in the explanation of some field observations and in the design of applied experiments.

Materials and Methods

For the study of population increase, sugar beets growing in sand in 6-inch pots were infected by adding 100 larvae per pot. The plants were divided into 4 lots of 10 plants and one lot was harvested every 6 weeks; cysts and white females were separated from roots and sand, screened, and collected for counting. Full and empty cysts were separated by picking before counting.

For studies with white females, young sugar beet plants growing vigorously in sand were infested with freshly hatched second stage larvae of *Heterodera schachtii*. After 36 days the plants were harvested and the roots and sand in which they were grown were thoroughly washed to collect the white females and cysts. White females without egg masses were selected from this source. Ivory or darker-colored females as well as cysts were discarded. The white females as well as other batches of cysts were tested for hatch response to a lyophilized material containing hatching factor. The cysts were used to confirm the activity of the hatch solution. The cysts and females were suspended at the surface of the solution by stainless steel screens in embryo dishes containing 2.5 ml. active solution. Each series containing six replicates of 75 white females or cysts was incubated in a humidity box at 25° C.[3] After collection of the larvae, the active solution was replaced.

[1] Research funds for this study were contributed in part by the Beet Sugar Development Foundation.

[2] Assistant Research Nematologist, Department of Plant Nematology, University of California, Davis, California.

[3] For practical considerations more of the brown cysts were used but the curves in Figure 2 have been corrected to 75. The white females used for hatch tests became ivory colored by the end of the experiment.

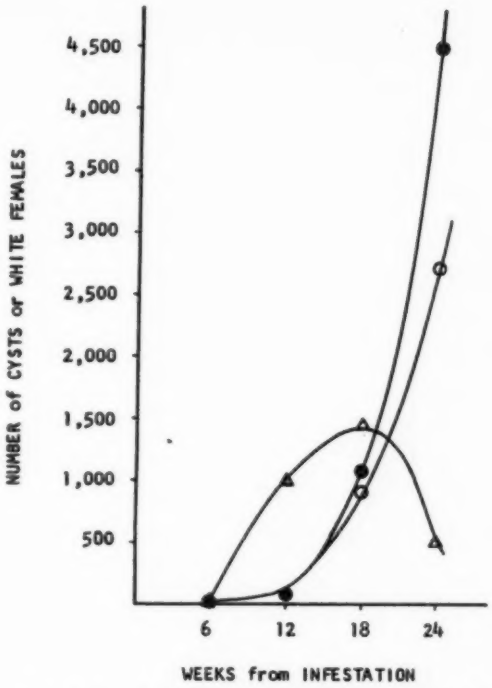

Figure 1.—Population increase of *Heterodera schachtii* Schm. from an initial infestation of 100 larvae. The solid circle represents the average of the total number of cysts; the open circle represents the average number of fuller cysts; the triangle represents the average number of white females.

Results and Discussion

Population increase

The curve described by cyst numbers is exponential as one would expect (Figure 1). With less than a hundred larvae which infected each plant and good growing conditions, a high population (white females plus cysts) developed in about the time period of two generations. This then explains how, with low populations in areas where the growing season permits less than two generations, it may be possible to employ shorter rotation periods safely, provided the nematode attrition rate is high enough. Effective control by rotation is dependent upon initial infestation as well as the rates of increase and decrease of the population. (For example, a similar experiment with about twice the initial infestation was found to give an additional fifty-fold increase in cysts in 24 weeks.)

In 24 weeks there are an appreciable number of cysts which are not full; viz., the difference between the two curves—dots, circles. Since cyst walls do not decompose readily, there is an inherent danger in the use of cyst counts as a population density indicator. In a heavily infested field on the order of 90% of the cysts can be empty (13); in addition there is the confounding problem of full cysts with non-viable contents resulting, for example, from fumigation, drying, etc. A simple unqualified cyst count is likely to be meaningless as an estimate of population potential.

The remaining curve clearly indicates the danger of incautious use of a white female count as a population indicator. It can be seen, for example, that a count of 500 can either comprise over 95 percent of the potential (inclining portion of curve) or less than 16 percent (declining portion of curve).[4] The steepness of the gradient also shows that the growth period is an important factor. The evidence here presented serves as a reminder of the wide variability of which the animal is capable.

With respect to field problems, it can be said with certainty that, if sugar beet nematode cyst counts can be made from field samples, then at some time *Heterodera schachtii* was present and may still be present; if white female counts can be made, *H. schachtii* is present. Any estimates of population potential based on such counts must be interpreted with extreme caution since they can be misleading. A proper population estimate (neglecting the problems in effective sampling for the purposes of this discussion) would require the determination of the viable contents of cysts and females. The criteria for a laboratory determination of viability are as yet poorly defined but a reasonable evaluation can be made by an emergence assay followed by cyst rupture and estimate of the viable remainder after the method of Feldmesser and Feder (4). The caution noted previously of the influence of environmental history (which, for practical considerations includes the environment from the time the sample leaves the field and the assay is made) upon results of emergence assays and "egg" determinations must be stressed. The use of this approach in Europe, however, testifies to its practicality (6).

Hatch response of white females

It had been observed that fresh cysts obtained from a growing bed gave very high water hatches when nearly all such cysts could

[4] Potential can be considered a summation of full cysts and females since viability is assured.

be at most 2 to 3 generations old (initial infestation from larvae). In view of the population increase curve, it was of interest to investigate the hatch response of white females. The literature on this point appears to contain conflicting views. According to Goodey (8), Chatin (1) concluded that "if the eggs were destined to be set free immediately the wall broke down and liberated them. If, on the other hand, they were to remain within the body, the wall of the latter became changed to a brown cyst . . ."

Fuchs (7) concluded that throughout the summer months the majority of females remained white and after dropping off from the roots of the host, liberated larvae at once which then infected fresh roots and only towards autumn did brown cysts form in quantity and retain their eggs. Triffitt (12) reported that larvae are not set free from cysts until the latter have turned brown. The first larvae issued about one month after the removal of the parent worms from the roots. A similar conclusion had been suggested earlier by Sengbusch (10) according to Filipjev and Schuurmans Stekhoven (5).

Presumably, the report of Thorne (11), that the eggs deposited in the gelatinous matrix at the posterior end of the female hatch readily, was taken into account. More recently, Franklin (6) and Christie (2) have suggested that though many larvae in eggs can remain dormant within the cysts for many years, some are capable of hatching as soon as they are fully developed. This remark introduces a further complication as to the meaning of "fully developed," since the term was neither qualified nor explained.

It is obvious that there is considerable hatch of larvae from the white females used in this experiment (Figure 2). It should also be apparent that there is a significant increase in hatch due to an active component in the hatch factor material. Though the percent increase in emergence is the same or less than that of the cyst controls, the increase in number of larvae emerging is greater than the total hatch of the best cyst trial.

The use of trap cropping as a control procedure has been considered risky. The hatching obtained with white females serves to confirm the hazards of the practice. In the use of any but immune crops the period of plant growth may be too short to make the trap crop useful.

Of equal importance for assay purposes is that in the interval examined, emergence is a linear function of time. Practically, it is far simpler to relate linear functions than complex functions. The linear curve indicates that emergence is independent of potential, i.e., not a function of hatchable larvae; either there is an excess of hatchable larvae initially or they mature to this state during the experiment at a rate greater than emergence so

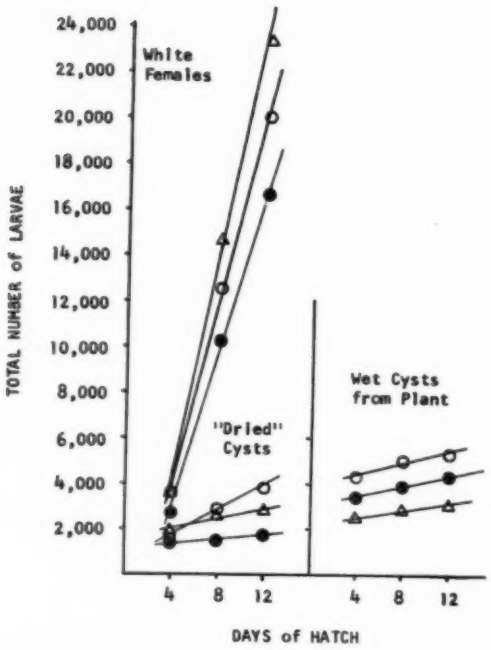

Figure 2.—The hatch response of 75 *Heterodera schachtii* Schm. white females and cysts from different sources. The solid circle represents the average water hatch; the open circle represents the average response to lyophilized sugar beet leachings at 1.0 mg/ml; the triangle represents the average response to leached material at 0.04 mg/ml.

that the potential pool of hatchable larvae in white females is not limiting. In the case of cysts the larvae presumably have had ample time for maturation so that the hatchable larvae potential could not be limiting. The hatching from white females is reflected as a maximum with increasing concentration of active component. The response reflected by the wet cysts from plants is a minimum, whereas the response of the "dried" cysts is still different.

It appears difficult to reconcile these observations with the report of Jones (9) that "A period of maturation appears to be necessary before eggs are fully sensitive to hatching stimuli," and Duggan (3) that a period of four weeks for maturation was necessary for cysts of *H. schachtii* before they were capable of hatching. The conclusions of Jones or Duggan are based on indirect evidence perhaps interpretable in other ways. The hatch tests

reported here were conducted under artificial conditions but it seems unlikely that an egg able to hatch from a white female detached from a root would be unable to hatch from a female attached to a root. More likely the sequence of environmental conditions during the development of the animals preceding emergence effect the observed responses. The apparent disagreement is an indication of our poor understanding of the factors governing hatching.

In this report the hatch-active factor in all cases is of the same batch and at the same concentration and the cysts and females are of common ancestry; therefore, one must conclude that the environmental history of the animals previous to testing is in part responsible for the differences in emergence. This being the case, then caution need be exercised in the comparison of hatch data from cysts of different origins or in the interpretation of occasional hatch tests with an unstandardized batch of cysts.

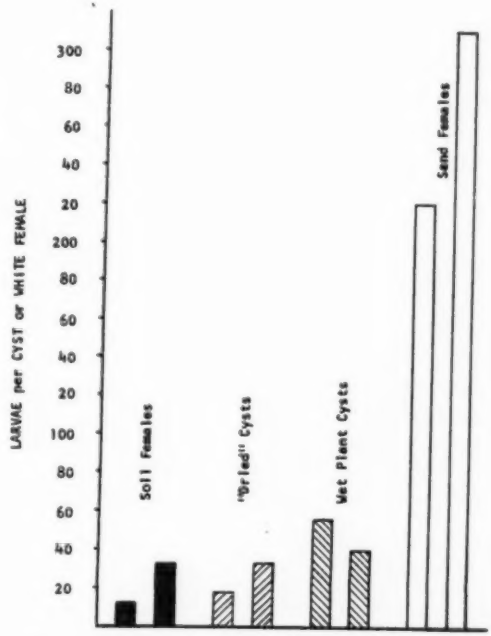

Figure 3.—The total larval hatch response per white female from two sources and per cyst from two sources after 12 days. Left bar, the response with water; right bar, the response to lyophilized leaching at 0.04 mg/ml.

In fact, there is no definite assurance that the reaction resulting in an increased hatch response is the same in white females as in wet cysts from a plant, i.e., the agent that is limiting may not be the same. In such a case the use of animals whose environmental history is uncertain could lead only to disaster. Figure 3 illustrates these points. The total number of larvae emerging in 12 days with two concentrations of active factor, namely 0.0 and 0.04 mg/ml. The hatch from another batch of white females of undetermined age obtained from soil is also shown. The results are quite different which, in view of the previous discussion, could be expected.

Summary

The population increase of *Heterodera schachtii* beginning with the addition of 100 infective second stage larvae to each pot of 4 lots of growing sugar beets is followed by harvesting a lot at 6-week intervals over a 24 week period. Sand and roots were washed, screened, and counts were made of total cysts, full cysts and white females. Cyst numbers increase exponentially and empty cysts begin to appear about the 15th week. The numbers of white females increase to a maximum then decrease during the 24 week interval.

White females less than 36 days old and with no visible egg masses were found to hatch profusely in distilled water. Immersion of the white females in lyophilized sugar beet leachings was observed to stimulate increased hatching over water controls.

Literature Cited

(1) CHATIN, J. 1887. Sur les cystes bruns de l'anguillule de betterane. C. R. Ac. Sci. CV: 130-132.

(2) CHRISTIE, JESSE R. 1959. Plant nematodes—their bionomics and control. Agric. Exp. Sta., Univ. of Florida, Gainesville, Florida, p. 89.

(3) DUGGAN, J. J. 1959. On the number of generations of beet eelworm, *Heterodera schachtii* Schm. produced in a year. Nematologica 4 (4) : 241-244.

(4) FELDMESSER, J. and FEDER, W. A. 1954. Maintaining and determining viability of nematodes in vitro. Soil Sci. Fla. Proc. 14: 154-157.

(5) FILIPJEV, I. N. and SCHUURMANS STEKHOVEN, J. H., JR. 1941. A manual of Agricultural Helminthology. E. J. Brill, Leiden, p. 520.

(6) FRANKLIN, MARY T. 1951. The cyst-forming species of *Heterodera*. Commonwealth Agricultural Bureaux, Fornham Royal, Bucks, England, p. 18.

(7) FUCHS, O. 1911. Beitrage zur Biologie der Rubennematoden, *Heterodera schachtii*. Z. landev. Vchsw. Ost. XIV: 923-949.

(8) GOODEY, T. 1933. Plant Parasitic Nematodes, E. P. Dutton & Co., New York, p. 132.

(9) JONES, F. G. W. 1956. Soil populations of beet eelworm (*Heterodera schachtii* Schm.) in relation to cropping. II Microplot and field plot results. Ann. Appl. Biol. 44 (1): 25-56.

(10) SENGBUSCH, R. 1927. Beitrage zur Biologie der Rubennematoden, *Heterodera schachtii*. Zeit. Pflanzenkrankh. 37: 86-102.

(11) THORNE, G. 1926. U. S. Dept. of Agri. Farmers Bull. No. 1514.

(12) TRIFFITT, M. J. 1930. Observations on the Life-cycle of *Heterodera schachtii*. J. Helm. VIII (4): 185-196.

(13) VIGLIERCHIO, DAVID R. 1959. Collection and selection of cysts of the sugar beet nematode, *Heterodera schachtii*. Jour. Amer. Soc. Sug. Beet Tech. 10 (4): 318-329.

Preparation of Melibiase-Invertase for Sugar Beet Molasses Assay of Raffinose

D. D. Leethem and R. E. Hallbeck[1]

Received for publication February 12, 1960

Introduction

The quantitative determination of sucrose in the presence of raffinose, in beet sugar factory materials, is considered most reliable when based upon methods involving the use of the enzymes invertase and melibiase. A commercial source of the latter enzyme could not be found in recent years.

Since suitable brewer's yeast, bottom fermenting, could not be obtained in quantity, a procedure for culturing a selected brewer's yeast in the laboratory was developed. The source of this selected yeast, and the harvest of its cultured yeast cells are described.

Extraction of invertase-melibiase from the yeast cells was performed according to the method described by Reynolds (2)[2].

Description of Yeast

A pure culture of the yeast *Saccharomyces carlsbergensis,* collection number C-126, was obtained from the Department of Food Science, University of California at Davis. This yeast was selected because of its ability to produce melibiase-invertase.

Procedures

The preparation of melibiase was begun with yeast inoculation into a growth medium. The yeast was cultured about four days and then harvested by filtration and the filtrate discarded. The yeast cells were autolysed in toluene. The autolysate was filtered and rinsed. The residue was discarded and the filtrate containing the enzymes was purified and concentrated by ultrafiltration. The melibiase concentrate was vacuum dried and the activity determined.

Laboratory Culture of Yeast

The yeast *S. carlsbergensis* was cultured in a 20 liter Pyrex bottle which contained 10½ liters of medium and yeast. The culture bottles, medium, and all accessories which could be the source of microbial contamination, were sterilized either by hot, dry air or by autoclaving. A modified Sabouraud medium at pH 4.8 and 7.6 rds was used for yeast cultivation.

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[1] Food Technologist and Assistant Head Research Chemist, Spreckels Sugar Company, Woodland, California.

[2] Numbers in parentheses refer to literature cited.

Table 1.—Yeast Growth Medium.

Constituent	Gms./Liter of Basal Nutrient
Sucrose	40.0
Raffinose	10.0
Dextrose	10.0
Polypeptone (Difco)	10.0
Yeast Extract (Difco)	8.0
Betalin (Lilly) 1:100	10.0 ml.
Basal Nutrient	**Gms/Liter, in Water**
$Ca(NO_3)2 \cdot 4H_2O$	1.12
KH_2PO_4	2.45
$MgSO_4 \cdot 7H_2O$	3.70
Micro-Enrichment Solution	**Mg/Liter, in Water**
(Use 1 ml. for each liter of Basal Nutrient)	
H_3BO_3	600
$MnCl_2 \cdot 4H_2O$	400
$ZnSO_4$	50
$H_2MoO_4 \cdot 4H_2O$	20
$Cu(Ac)_2$	20
$Co(Ac)_2$	20
$FeSO_4$	7,000

Balab No. 613 antifoam is added ½ drop per liter of growth medium nutrient.

To reduce the time required for maximum vigorous growth in the large culture, a "mother culture" was first prepared in a one liter flask. About 500 ml. of growth medium were used in the "mother culture" which was inoculated with a loop of the yeast. The mother culture was incubated for 24 hours at room temperature and then carefully poured into the 20 liter culture bottle together with 10 liters of medium. Then all the accessories were attached to the culture bottle and aeration was begun.

After eight hours of growth the nutrient supplement was fed to the culture at a rate which maintained the culture between pH 3.5 and pH 4.8 and the sugar concentration above 4.0 rds.

Table 2.—Nutrient Supplement: (pH 4.8, rds 20.8).

Sucrose	440 g.
Raffinose	200 g.
Nutrient solution	3 liters

The practical maximum yeast cell development usually required four days. Twelve hours before the yeast cells were to be harvested, aeration was stopped to allow the cells to settle thus facilitating the harvest.

Filtration of Yeast Cells from Growth Media

When the yeast cells were ready for harvest they were separated from the growth medium by vacuum filtration on a Buchner funnel. Filtration was simplified by carefully siphoning off the medium above the cells in the culture bottle. The medium could be rapidly filtered. Then the yeast cells were poured from the culture bottle into the Buchner funnel and filtered as dry as possible. The yeast cells were then ready for autolysis, and the filtrate was discarded. A yield of about 400 grams wet weight was obtained.

The National Bureau of Standards (1) method for preparation of melibiase extract from brewer's yeast was followed to obtain an enzyme concentrate.

Drying Melibiase Concentrate

The ultrafiltered melibiase-invertase concentrate was placed in a 500 ml vacuum flask which was immersed in a water bath maintained at 35° C. Gentle magnetic stirring was used until the enzyme concentrate became too viscous for the stirrer to operate. Then the stirring bar was removed and the drying continued without agitation. About 12 hours were required for the final drying after which the dried melibiase-invertase is ready for use or storage. The yield was about 2 gms of dried concentrate.

Melibiase-Invertase Activity Determinations

The activity of an enzyme is generally expressed in terms of the velocity of its specific reaction with a definite substrate.

The activity of melibiase and invertase were determined by observing the changes in optical rotation of melibiose and sucrose solutions through a period of time.

The hydrolysis velocity constant was calculated by the following formula:

$$k = \frac{1}{t} \times \log \frac{P_i - P_f}{P_t - P_f}$$

Where k = the hydrolysis velocity constant
t = period of hydrolysis in minutes
P_i = initial polarization reading
P_f = final polarization reading
P_t = polarization reading at time t
$k = 0.0040$ for melibiase activity
$k = 0.0225$ for invertase activity

Another indication of melibiase-invertase activity can be obtained simply by taking polariscopic readings of a raffinose

solution at zero time and again after over-night hydrolysis. The ability of the enzymes to completely hydrolyze the standard amount of raffinose is a measure of activity which can be conveniently applied to sugar beet molasses analyses.

Raffinose hydrate, 1.35 gms, in water to make a volume of 50 ml was hydrolyzed overnight by 2 ml of a 5% solution of the dried melibiase-invertase concentrate. This is sufficient activity for the analysis of beet molasses wherein raffinose/100 solids is 6%.

Summary

The cultivation of *Saccharomyces carlsbergensis* in the laboratory for the production of melibiase-invertase has been described. An invertase-melibiase product satisfactory for the hydrolysis of raffinose was obtained from the laboratory grown yeast by following published procedures for the separation and purification of the enzyme.

Literature Cited

(1) United States Department of Commerce, National Bureau of Standards, 1942. Circular C 440 Polarimetry, Saccharimetry, and The Sugars, Pp. 147, 164.

(2) Reynolds, F. W. 1924. The rapid analysis of sugar, purification and concentration of enzyme solutions. Ind. and Engr. Chem. 16: 169, 172.

Comparison of Statistical Designs for a Large Number of Entries of Sugar Beet Strains

H. L. Bush and R. K. Oldemeyer[1]

Received for publication May 24, 1960

Field testing of a large number of entries presents a problem when the results are interpreted, since the results may reflect soil variation from the rather large area which is covered by each replicate.

The familiar Lattice Designs[2] were developed for the purpose of minimizing heterogeneity by means of statistical analyses. However, difficulties arise with these designs when seed quantities are low or of poor quality so that there may be some variation in the possible number of replicates for some lots. Also, due to poor stands, some plots may be lost, necessitating the calculation of missing plot values.

In an attempt to provide a scheme which was not as fixed in its basic design, whereby the number of entries or number of plots for each entry was not vital to the design, a scheme[3] was devised so that 2 plots of a "control" or check variety were included in each block which also contained 10 entries of the strains under consideration. Results for each plot were then calculated in "% of check" before being averaged. The Standard Error value obtained from the check variety could be taken as an estimation of the variability that might be applied to all entries. This design, as it has been used, has given highly satisfactory results. However, as the number of entries increased, it became evident that it was inefficient from the land-usage standpoint, as well as labor costs, since approximately 17% of the plots of a 10 entry block were planted to the control variety.

It was decided in 1958 to determine the precision of this method as compared with the Lattice Design, since the Control Block Design appeared to be getting unwieldy. In order to make this comparison, a Simple Lattice Design composed of 100 top-cross lines in 4 replicates was set up. Two plots of the control variety were then added in a random location within each block of the lattice. The data were analyzed according to both schemes.

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[1] Statistician-Agronomist and Director of Seed Development, respectively, Great Western Sugar Company, Longmont, Colorado.

[2] Cochran, Wm. G., and Cox, Gertrude M. 1957. Experimental Designs, 2nd Edition, John Wiley and Sons, Inc., New York 1, N. Y.

[3] Credit is given to Dr. LeRoy Powers, U. S. Department of Agriculture, for suggesting this type of design.

The performance of the top crosses calculated from the control block design deviated, plus or minus, from the performance calculated by the lattice design. Tables 1 and 2 summarize the deviations for yield of roots and sugar content. The bulk (83) of the observations do not deviate more than 5% from one method to the other while 15 of the entries give the same results (within 0.5%) by the two methods. When this is considered in the light of the wide range of performance of the entries, 65-135% of the check, this deviation is of minor importance.

Table 1.—Root Yields for Top-Crosses as Indicated by the Lattice Design Compared with Those Obtained by Control Block Design.

Top-Crosses	Deviation of Block from Lattice Results
(No.)	(%)
1	−10
1	−9
2	−8
2	−7
3	−6
5	−5
4	−4
6	−3
8	−2
9	−1
15	0
8	+1
7	+2
8	+3
6	+4
7	+5
1	+6
3	+7
2	+8
1	+9
0	+10
1	+11

Using the same method of comparison for sugar content, Table 2, the deviation is even smaller than for yield of roots. The range of performance for this character was 80-118% of the check. In this case, 96 of the observations deviated 2.5% or less for the two designs and 24 of the entries were the same (within 0.25%).

Inasmuch as gross sugar production is a function of root yield and sugar content, a table for comparing the two methods is not presented for this character.

The two designs have also been compared in another manner whereby standards have been set for the inbred top crosses, expressed in percent of the check strain, of 104% or less for discontinuance of the inbred line, 105-114% for retesting and 115%

Table 2.—Sugar Content for Top-Crosses as Indicated by the Lattice Design Compared with Those Obtained by Control Block Design.

Top-Crosses	Deviation of Block from Lattice Results
(No.)	(%)
1	—3.0
5	—2.5
2	—2.0
6	—1.5
12	—1.0
14	—0.5
24	0
12	+0.5
5	+1.0
6	+1.5
4	+2.0
6	+2.5
1	+3.0
0	+3.5
0	+4.0
2	+4.5

or more for increase. These standards apply particularly to yield of roots and gross sugar. Comparing the data from the two designs for root yield, 9 entries would indicate a retest by one design and discontinuance by the other. Considering gross sugar, 8 inbreds would be shelved by one design and retested by the other; 5 would be increased by one scheme and retested by the other. In no instance for either root or gross sugar yield was the deviation great enough that an inbred would be discontinued as the result of one design and increased as the result of the other, which would indicate that we would not discontinue any of the better lines by either scheme.

The Lattice Design was used for the 1959 tests and these data indicate a high degree of reliability as judged from the similarity of results obtained from testing related lines.

The authors have concluded that the Lattice Design is sufficiently accurate to be used in testing the bulk of the hybrids, and due to the considerable saving in number of plots to be tested, is probably preferable to the Control Block Design. For testing hybrids of questionable germination or those which are limited in seed, the Control Block Design will continue to be used.

In Vitro Inoculation of Sugar Beet Seedlings with Aphanomyces Cochlioides Drechs.[1]

H. S. MacWithey

Received for publication June 13, 1960

Introduction

Pathogenesis in *A. cochlioides* Drechs. is dependent to a great extent upon high soil moisture. Free water is necessary not only for zoosporangium and zoospore formation, but also for movement of the zoospores through the soil and infection of the host plant (1, 2)[2]. If moisture should become limiting during this period of motility and infection, the effective exposure time of the sugar beet to infection and the relative number of infections sustained by the seedlings would be reduced. Furthermore, the number of infections sustained by the seedlings might show some relationship to the severity of black root symptom expression. To investigate these proposed relationships, *in vitro* inoculations were performed using standardized concentrations of zoospores and definite times of exposure of the sugar beet seedlings to the zoospore inoculum.

Materials and Methods

The culture of *A. cochlioides* used in this investigation was obtained from diseased seedlings growing in artificially infested field soil. For zoospore production, cultures of *A. cochlioides* were grown for five days in 125 ml. flasks containing 50 ml. of a liquid nutrient medium. This medium consisted of Difco-Bacto-Peptone, 1.0 g., Difco-Bacto-Yeast Extract, 1.0 g., potassium mono-basic phosphate, 0.5 g., magnesium sulfate, 0.5 g., and dextrose, 2.0 g., dissolved in 1000 ml. of distilled water. At the end of the growth period, the nutrient medium was decanted, the culture was washed with sterile distilled water and then resuspended in 50 ml. of sterile distilled water and placed at 20° C. for 24 hours. Zoospore concentrations were determined using a Levy-Hausser counting chamber. Sugar beet seedlings, used in these tests, were grown in steam-sterilized soil from G.W.359 segmented seed, treated with N. I. Ceresan. All isolations from inoculated sugar beet seedlings were made on corn meal agar medium.

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[1] Contribution from Montana State College, Agricultural Experiment Station, Bozeman, Montana. Paper No. 493.

[2] Numbers in parentheses refer to literature cited.

Experimental Results

Zoospore concentrations, able to cause uniform infection within a 24-hour period, were determined in the following manner. Zoospore suspensions containing 20, 200, 2,000 20,000 and 200,000 zoospores/ml. were made up in sterile distilled water and used for inoculum. Four-day-old sugar beet seedlings, washed free of soil, were laid in culture dishes containing 20 ml. of zoospore suspension. Four dishes of twenty seedlings each were prepared for each inoculum concentration and maintained at 20° C. for 24 hours. Seedlings in sterile distilled water were used as checks. Following this exposure to inoculum, the seedlings were removed from the zoospore suspensions and washed vigorously in three changes of distilled water. A one-centimeter section of hypocotyl was removed from each seedling and transferred to a corn meal agar plate. Mycelium of *A. cochlioides*, growing from the infected tissue, was visible in 24 to 48 hours. All hypocotyl sections from seedlings exposed to concentrations of 20,000 zoospores/ml. or greater were infected. At the lesser zoospore concentrations of 2,000, 200, 20 and 0, infection decreased to 80, 10, 2.5 and 0 percent respectively (Table 1).

Table 1.—Infection of Sugar Beet Seedlings Exposed for 24 Hours to Various Concentrations of Zoospores of *A. cochlioides*.

Number of Zoospores/ml.	Hypocotyl Infection	
	Number	Percent
0	0	0.0
20	2	2.5
200	8	10.0
2,000	64	80.0
20,000	80	100.0
200,000	80	100.0

Four-day-old sugar beet seedlings were next exposed to suspensions containing 20,000 zoospores/ml. for 2, 4, 8, 16 and 24-hour periods. Four culture dishes, containing 20 seedlings per dish were prepared for each time period. Inoculation and isolation procedures were identical to those in the previous experiment. Seedlings exposed for 2, 4, 8, and 16 hours were placed in dishes of distilled water after washing and plated at the same time as the 24-hour treatment. This provided the same period of time for development of all infections. In all treatments, 80 percent or more of the seedlings exposed to the inoculum were infected (Table 2).

Table 2.—Infection of Sugar Beet Seedlings Exposed to a Standard Zoospore Inoculum of *A. cochlioides* for Various Periods of Time.

Hours of Exposure to Inoculum	Hypocotyl Infection	
	Number	Percent
0	0	0.0
2	64	80.0
4	66	82.5
8	78	97.5
16	80	100.0
24	80	100.0

The mycelial growth produced from infected hypocotyl tissue was progressively more dense as time of exposure increased. This indicated that a greater number of infections had taken place with a corresponding increase in time.

To determine if increase in time of exposure to inoculum would cause an increase in severity of disease symptoms, inoculated four-day-old sugar beet seedlings were transplanted to bench plots of steam-sterilized soil in the greenhouse. Times of exposure to inoculum, inoculation and isolation procedures were the same as above. Disease readings were made eight days after transplanting, after which the seedlings were removed from the soil and the entire hypocotyl excised and plated on corn meal agar.

Seedlings exposed to the zoospore inoculum for 16 and 24 hours showed, in addition to the usual hypocotyl blackening, wilting and collapse of the hypocotyl tissue. Wilting was also evident in the 8-hour treatment, but most of the seedlings showed only a severe hypocotyl discoloration. In seedlings exposed for 2- and 4-hour periods, hypocotyl blackening was not as severe as in the 8-hour treatment and the majority of the seedlings displayed no above-ground symptoms of disease. However, isolations from these symptomless plants showed Aphanomyces to be present in most cases in a slightly discolored area, 1 to 3 mm. long, at the base of the hypocotyl. To distinguish between these plants and plants showing definite above-ground symptoms, two infection categories were set up. Apparent infection values were based on the percentage of seedlings showing above-ground symptoms. Total infection values were based on the percentage of seedlings from which *A. cochlioides* was isolated (Table 3).

Discussion

This investigation has shown that differences in time of exposure of sugar beet seedlings to a standard zoospore inocumum

Table 3.—Infection and Symptom Expression in Sugar Beet Seedlings Exposed to a Standard Zoospore Inoculum of *A. cochlioides* for Various Periods of Time.

Hours of Exposure to Inoculum	Apparent Infection		Total Infection	
	Number	Percent	Number	Percent
0	0	0.0	0	0.0
2	16	20.0	78	97.5
4	30	37.5	78	97.5
8	62	77.5	80	100.0
16	78	97.5	80	100.0
24	80	100.0	80	100.0

of *A. cochlioides* result in different intensities of disease expression. These differences in disease expression are believed to be a function of the number of infections sustained by the seedlings. Although it was not possible to determine the actual number of infections, it appears that a certain minimum number are required to induce typical black root symptoms. On the other hand, there is apparently a level above which the infections are so numerous as to cause wilting and rapid death of the seedling. Such symptoms suggest penetration of the normally resistant vascular tissue or production of toxic products resulting from the large number of infections.

Inoculation of sugar beet seedlings *in vitro* allows close control of inoculum concentration and environmental factors affecting the infection process. Such control is not possible when soil is used as the infection medium. This technique could be useful in investigations of nutritional and biological factors affecting infection and disease development. Also by providing uniformly infected seedlings, a more accurate screening of lines of sugar beets for resistance to *A. cochlioides* would be possible.

Literature Cited

(1) DRECHSLER, CHARLES. 1929. The beet water mold and several related root parasites. Jour. Agr. Res. 38: 309-361.

(2) MCKEEN, W. E. 1949. A study of sugar beet root rot in southern Ontario. Can. Jour. Res. C. 27: 284-311.

An Analysis of Production Practices of Sugar Beet Farmers in Michigan, 1958[1]

J. F. Davis and M. G. Frakes[2]

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Research in methods of reducing spring labor requirements for sugar beets has been given a high priority rating by industry and experiment station workers. In order to coordinate efforts of the two groups a cooperative project was initiated in 1957 between various departments of the Michigan Agricultural Experiment Station and the Farmers and Manufacturers Beet Sugar Association. The objectives of this project were to investigate ways of minimizing spring labor requirements in sugar beet production. As part of this research program, the Soil Science Department and the Agricultural Research Department of the Michigan Sugar Company conducted a study of production practices of sugar beet farmers in Michigan. Practices from approximately 3,000 fields which consisted of 49,000 acres of sugar beets, were obtained by fieldmen. The data were coded and punched on IBM cards and then analyzed through the facilities of Michigan State University under the direction of personnel of the Soil Science Department.

These data are for but one year, but they do represent cultural practices from approximately 70 percent of the acreage of sugar beets in Michigan. It was believed that a description of the methodology involved in this study would be of interest to research personnel of both experiment stations and industry. It also demonstrates the advantages of a cooperative project between two interested organizations, the Company and the Experiment Station.

Procedure

Standard IBM cards were used and the following items coded on the cards:

1. District. Five districts.
2. Year. 1957 to 1966.
3. Fieldmen. Thirty-six fieldmen.
4. Soil type. Mineral, muck, combinations.
5. Tile drained. Yes, no.
6. Surface drained. Yes, no.

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[1] Contribution of the Soil Science Department of the Michigan Agricultural Experiment Station, Michigan State University, and the Michigan Sugar Company, Saginaw, Michigan. Authorized for publication by the Director as Journal Article No. 2579 of the Michigan Agricultural Experiment Station, East Lansing, Michigan.

[2] Professor of Soil Science, Michigan State University and Agricultural Research Director, Michigan Sugar Company, respectively.

7. Preceding crop. Corn, small grain, beans, beets, first year seeding, established seeding, potatoes, mint, others.
8. Crop two years previous. Corn, small grain, beans, beets, alfalfa or clover, potatoes, mint, others.
9. Crop three years previous. Corn, small grain, beans, beets, alfalfa or clover, potatoes, mint, others.
10. Alfalfa or Clover. Preceding year, two years previous, three years previous, and no alfalfa or clover.
11. Plowing practices. Fall, spring, not plowed.
12. Depth of plowing (inches). Less than 3.9, 4 to 5.9, 6 to 7.9, 8 to 9.9, 10 to 11.9, 12 and over.
13. Times worked after plowing. One, two, three, four, five, more than five, none.
14. Days between working of soil and planting. Less than one, one, two, three, four, five, over five.
15. Pounds of fertilizer plowed down. None, 1-199, 200-299, 300-399, 400 to 499, 500 to 599, 600 to 699, 700 to 799, 800 or more.
16. Pounds of fertilizer broadcast. None, 1-199, 200-299, 300-399, 400 to 499, 500 to 599, 600 to 699, 700 to 799, 800 or more.
17. Pounds of fertilizer applied with a grain drill. None, 1-99, 100-199, 200-299, 300 to 399, 400 to 499, 500 to 599, 600 to 699, 700 or more.
18. Total pounds of fertilizer used. None, 1-199, 200-399, 400-599, 600-799, 800 to 999, 1000 to 1199, 1200 to 1399, 1400 or more.
19. Analyses of fertilizer plowed down. None, x-2-1, x-1-1, x-1-2, x-1-3, 0-2-1, 0-1-1, 0-1-2, 0-1-3.
20. Analyses of fertilizer broadcast. None, x-2-1, x-1-1, x-1-2, x-1-3, 0-2-1, 0-1-1, 0-1-2, 0-1-3.
21. Analyses of fertilizer with drill. None, x-2-1, x-1-1, x-1-2, x-1-3, 0-2-1, 0-1-1, 0-1-2, 0-1-3.
22. Pounds of actual nitrogen sidedressed. None, 1-39, 40-49, 50-59, 60-69, 70-79, 80-89, 90-99, 100 or more per acre.
23. Total pounds nitrogen per acre used in fertilizer. None, 0-39, 40-59, 60-79, 80-99, 100-119, 120-139, 140-159, 160 or more.
24. Total pounds phosphate (P_2O_5) used. None, less than 50, 50-99, 100-149, 150-199, 200-249, 250-299, 300-349, 349 or more per acre.
25. Total pounds potash (K_2O) used per acre. None, less than 50, 50-99, 100-149, 150-199, 200-249, 250-299, 300-349, 349 or more.

26. Pounds of seed per acre. 0-.9, 1.0-1.4, 1.5-1.9, 2.0-2.4, 2.5-2.9, 3.0-3.4, 3.5-3.9, 4.0-4.4, 4.5 or more.
27. Row width (inches). Less than 28, 28, 30, 32, 34, 36, 38, 40, more than 40.
28. Seed spacing (inches). Less than 1.4, 1.5-1.9, 2.0-2.4, 2.5-2.9, 3.0-3.4, 3.5-3.9, 4.0-4.4, 4.5-4.9, 5 or more.
29. Cultivating within two inches of the row. Yes or no.
30. Number of times cultivated. None, 1, 2, 3, 4, 5, 6, 7, over 7.
31. Date planted. Before April 1, April 1-11, April 12-23, April 24-May 6, May 7-18, May 19-30, May 31 to June 11, June 12-23, after June 23.
32. Drill used. Palsgrove, John Deere flex, John Deere 64, International, other.
33. Type of labor. Own labor, Nationals, Texans, Combination, and Local.
34. Cost per acre thinning.
35. Cost per acre hoeing.
36. Total cost per acre.
37. Chemical weed control. Yes, no.
38. Mechanical stand reduction. Yes, no.
39. Date harvested. Before October 1, Oct. 1-7, Oct. 8-15, Oct. 16-23, Oct. 24-30, Oct. 31-Nov. 7, Nov. 8-15, Nov. 16-23, after Nov. 23.
40. Number of acres harvested.
41. Total tons per acre.
42. Acres lost.
43. Reason for loss. Weather, weeds, disease, poor stand, soil bank, flood, other.
44. County. Twenty-two counties.
45. Township. 183 townships.

This coding required 66 out of 80 spaces available on a standard IBM card, thus leaving 14 spaces for other items.

Prior to coding on the cards the field forms were checked by the Agricultural Research Director for the company. The data were then recorded on a standard tabulation sheet provided for that purpose, and were then submitted to the Statistical Laboratory where the cards were punched and the various analyses desired run. A preliminary run of the following comparisons was made.

1. Number of acres and yields per acre as indicated by IBM analyses and company postharvest records.

2. Mineral versus muck soils.
3. Tile drainage.
4. The effect of preceding crops on yields.
5. The number of years beets were preceded by alfalfa.
6. Fall versus spring plowing.
7. Depth of plowing.
8. Number of times field was worked prior to planting.
9. Number of days between last time the soil was worked and planting.
10. Amount of fertilizer plowed down.
11. Amount of fertilizer broadcast before planting.
12. Fertilizer applied with a drill at planting time.
13. Total amount of fertilizer applied.
14. Nitrogen sidedressing.
15. Total amount of nitrogen applied.
16. Relationship of nitrogen application and time of plowing down alfalfa or clover.
17. Amount of phosphate applied.
18. Amount of potash applied.
19. Effect of PK ratio.
20. Date of planting.
21. Date of harvest.

There are several other comparisons that could be made. As a matter of illustration a few of the tables of data are cited. For example, in Table 1 the relationship of the number of acres and yield per acre as indicated by IBM analyses and company post-harvest records, 1958, are recorded. There are a few small discrepancies in the total acreage harvested between the two methods of analysis, particularly for the Alma, Caro, and Saginaw Dis-

Table 1.—Number of Acres and Yield per Acre as Indicated by IBM Analysis and Company Post-Harvest Records, 1958.

	IBM Analysis		Actual	
District	Acres	Tons/Acre	Acres	Tons/Acre
Alma (a)	4,226	14.2	4,155	14.4
Caro	10,772	16.5	10,452	16.6
Croswell	6,760	11.9	6,957	11.9
Saginaw	9,583	18.0	9,511	17.1
Sebewaing	17,449	16.1	17,760	15.8
Total	48,790	15.8	48,835	15.6

(a) Alma figures include acres of beets grown in the Lansing area.

tricts. The only major discrepancy is shown in the average yield per acre in the Saginaw District. In this case, the IBM analysis shows nine-tenths tons per acre more beets produced than the company's final figures. When considering this with the acreage harvested in relation to the entire acreage, the figures appear reasonably accurate.

The effect of tile drainage on the yield of beets also shows some interesting information. On the average, tile drained land produced 3.2 tons per acre more beets than did untiled land. However, there was quite a wide variation between districts as to the effect of tiling. The figures show that approximately 80 percent of the total beet acreage was tiled land and the greatest apparent benefit from tile drainage was in the Caro District where the beets produced on tiled land averaged 4.8 tons per acre more than those grown on land not tiled.

Table 2.—Yields of Beets as Affected by Tile Drainage, 1958.

District	Tile Drained	Acres	Tons/Acre	Difference
Alma	Yes	2,750	14.8	1.3
	No	341	13.5	
Caro	Yes	9,047	17.2	4.8
	No	1,643	12.4	
Croswell	Yes	1,578	13.4	1.7
	No	4,285	11.7	
Saginaw	Yes	7,618	18.5	3.7
	No	1,352	14.8	
Sebewaing	Yes	14,131	16.2	0.2
	No	3,171	16.0	
Total	Yes	35,124	16.7	3.2
	No	10,771	13.5	

Another interesting comparison was obtained by the number of times the field was worked prior to planting. The trend generally occurring at all plant areas both individually and collectively indicates that the highest yields were obtained with either once or twice over the field before planting. This brings out the marked advantage of the minimum tillage idea in production of sugar beets. Not only are yields maintained at the highest levels, but there is a considerable advantage in the reduced expense involved in seedbed preparation.

Table 3.—Beet Yields as Affected by the Number of Times a Field Was Worked Prior to Planting, 1958.

Times Worked	Acres	Tons/Acre
One	10,316	16.8
Two	17,350	16.7
Three	9,237	15.2
Four	3,303	14.8
Five	947	14.2
Six	723	14.3
None	4,019	14.0

Another interesting comparison was obtained from the amount of phosphate applied. As the amount of phosphate increased, the average yield per acre increased, up to and including 350 pounds of P_2O_5 per acre. A more complete report of the results can be obtained from the Michigan State University Agricultural Experiment Station Quarterly Bulletin, Volume 42, No. 2, pages 401-421, 1959.

Table 4.—Beet Yields as Affected by the Amount of Phosphate Applied, 1958.

Pounds/Acre	Acres	Tons/Acre
Less than 50	2,463	14.3
50- 99	14,331	15.5
100-149	22,025	16.3
150-199	5,712	16.2
200-249	1,197	17.5
250-299	114	17.9
300-349	46	18.0
Over 349	6	17.2

It is realized that there is confounding of factors that should be considered in the interpretation of these results. For example, farmers on tile drained farms are more likely to be better farmers and, in general, better farmers tend to follow the better practices. However, this type of processing of data does provide some interesting trends. Furthermore, where acreages of less than 1,000 acres are involved, care should be taken in the degree in which the effect of this particular factor at this level of acreage is assessed.

While the data are for but one year, it is planned to continue this type of study, possibly not on a year-to-year basis but at various intervals and in this way a good comparison can be made

with changes in practices over time. The cost of this analysis is comparatively reasonable, particularly if the data are collected by the fieldmen as a standard procedure by the company. Apparently, most companies do keep some sort of record on each field of beets and after the data are collected this appears to be a satisfactory way of analyzing a large number of figures.

Extreme care should be taken in training personnel to provide accurate information. This, in itself, is good training for the fieldmen and it also provides a close check on the fieldmen's operation.

This study also provides an opportunity for combining data from year to year and studies of this kind should contribute much to the progress of sugar beet production.

Effect of Gibberellic Acid on Sucrose, Purity and Weight of Sugar Beets

DONALD F. PETERSON[1]

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Gibberellic acid has been reported to increase rate of growth in many plant species (5)[2]. Initial experiments with gibberellic acid on sugar beets were concerned with increasing the rate of seed stalk production (1). Following this, several workers reported the effect of gibberellic acid on sucrose and weight of the sugar beet root (2, 3, 4). Peterson (3) reported that applications of 100 and 500 p.p.m. of gibberellic acid reduced sucrose and tended to increase yield. Nelson and Wood (2) showed that a 10 p.p.m. application resulted in a root yield increase significant at the 1% level and a sugar production increase significant at the 5% level. Stout (4) concluded that gibberellic acid reduced sugar percentage and respiration rate of root tissue when applied to the growing points of large sugar beets in the field.

In our work previously reported (3), applications of gibberellic acid made in July, August and September, resulted in reduced sucrose content. It was our thought that by making applications of gibberellic acid earlier in the growing season, this sucrose reduction might be avoided. Also, earlier spring applications might increase leaf surface more rapidly during the longest days of the year, resulting in increased production. This paper reports results from such tests conducted in 1958 and 1959.

Materials and Methods

Applications of gibberellic acid were made in concentrations of 10, 100, and 500 p.p.m. at the rate of 12 gallons per acre. Each concentration was applied from one to four times with the second, third and fourth applications occurring 5, 10 and 15 days after the initial application was made on June 12. Thus, plots given the initial application received 10, 100 or 500 p.p.m. at the rate of 12 gallons per acre. A second set of plots was treated twice at 12 gallons per acre with concentrations of 10, 100 or 500 p.p.m. The third and fourth sets of plots were treated three and four times respectively with concentrations of 10, 100 or 500 p.p.m. of gibberellic acid at the rate of 12 gallons per acre.

The tests in both years were of randomized complete block design. Plot size was one row 25 feet long. There were six replications of each treatment. One guard row was planted between each plot to prevent adjacent plots from being affected

[1] Plant Breeder, Holly Sugar Corporation, Sheridan, Wyoming.

[2] Numbers in parentheses refer to literature cited.

at time of spraying. All treatments were made with a hand sprayer. The entire plot was harvested for yield and sucrose determination.

Experimental Results

Data for the two years was combined for analysis. The year by treatment interaction was not significant. Significant treatment differences were obtained for all characters studied. Table 1 gives the data for the treatments studied.

Table 1.—Performance Data for Plant Spray Treatments Using Gibberellic Acid at Sheridan, Wyoming, in 1958 and 1959.

Conc. P.P.M.[1]	No. of Applications	Sugar/Acre Lbs.	Beets/Acre Tons	Sucrose %	Thin Juice Purity %	Bolters %	Beets/100 Ft. Row
10	1	5553	18.7	14.9	93.5	.2	127
10	2	5346	17.7	15.1	93.1	.3	119
10	3	5319	17.8	14.9	93.3	.2	122
10	4	5213	17.6	14.8	93.1	.9	118
100	1	5420	18.0	15.0	92.7	.4	117
100	2	5210	17.6	14.8	92.9	.4	120
100	3	5307	18.0	14.7	93.2	1.3	122
100	4	5324	17.9	14.9	92.6	5.0	124
500	1	5194	17.8	14.6	92.4	4.0	122
500	2	4970	17.4	*14.3*[2]	92.3	7.5	121
500	3	*4556*[2]	*15.8*[2]	*14.3*	92.6	31.6	122
500	4	*4605*	*15.7*	14.7	*91.8*[2]	58.4	118
Check[3]		5272	17.8	14.8	92.9	0	117
L.S.D. (5%)		439	1.4	.5	.8		

[1] Gibberellic acid at the rate of 12 gallons per acre.
[2] Italicized values differ significantly from the check.
[3] No material applied.

Yield reductions occurred when three and four applications of 500 p.p.m. of gibberellic acid were applied. There was also an indication of sucrose reduction with two and three applications of 500 p.p.m. of gibberellic acid although the four applications of 500 p.p.m. treatment was equal to the check. These reductions are reflected in lower sugar per acre for the three and four 500 p.p.m. applications. Thin juice purity was lowered significantly with four applications of 500 p.p.m. of gibberellic acid. Bolting increased materially in the higher rates of treatment.

Summary

In 1958 and 1959 spray applications of gibberellic acid were made on sugar beet plants in the last half of June at Sheridan, Wyoming.

Single and multiple applications of 10 or 100 p.p.m. produced no crop response. Two, three and four applications of 500 p.p.m. tended to reduce tonnage, sucrose, sugar per acre and thin juice purity and to increase percent bolting. These data indicate that it is doubtful that gibberellic acid applied early in the growing season under the conditions of these tests will have a role in increasing sugar beet production.

Literature Cited

(1) GASKILL, J. O. 1957. A preliminary report on the use of gibberellic acid to hasten reproductive development in sugar beet seedlings. Jour. Amer. Soc. Sugar Beet Tech. IX (6): 521-528.

(2) NELSON, R. T. and WOOD, R. R. Studies of chemical treatments on sugar beets including gibberellic acid, 2,4-D, and maleic hydrazide. Jour. Amer. Soc. Sugar Beet Tech. X (1): 76-79.

(3) PETERSON, DONALD F. 1958. Effect of gibberellic acid on germination, sucrose, and yield of sugar beets. Jour. Amer. Soc. Sugar Beet Tech. X (1): 53-55.

(4) STOUT, MYRON. 1959. Some effects of gibberellic acid on the physiology of sugar beets. Jour. Amer. Soc. Sugar Beet Tech. X (4): 305-310.

(5) WITHWER, S. H. and BUKOVAC, M. J. 1958. The effects of gibberellin on economic crops. Econ. Botany XII (3): 213-255.

Use of Unit Block Selection Method for Yield, Sugar and Purity in Sugar Beets

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Low sucrose and low purities have been continuing problems in the California, Imperial Valley sugar beet producing area. Any varietal improvement to correct the problems would be most welcome and valuable to factory operation. In 1957, it was decided to act upon a suggestion of Dr. LeRoy Powers, Principal Geneticist, U. S. Department of Agriculture, to make a selection for high sucrose and high purity, utilizing the "Unit Block" selection method.

Materials and Methods

A field of beets planted to the commercial variety HC 1 on the Nelson Corral ranch near Brawley, California, was chosen for this work. The field was selected because of its apparent uniformity in stand, fertility and freedom from disease. About an acre was marked off into small blocks, each 20 feet long and 14 rows wide. The beets in each block were dug by hand and laid up on the ground. From each block 100 of the largest beets were selected for further testing. In all, 83 blocks were handled in this manner.

The 8300 beets were sent to Sheridan, Wyoming, to be tested for sucrose and purity. Mean sucrose and mean purity values were calculated for each block. Those roots whose sucrose and purity values exceeded their block means by the greatest percentage for both characters were selected for further testing. On this basis more than one root could be selected from a block or no roots could be selected from a block. Fifty-two roots were saved and put out in two groups for seed production at Sheridan in 1958. In group one the six roots that exceeded their block means by the greatest percentage for both sucrose and purity were included. The remaining 46 roots were planted in the second group.

Cuttings were made from each plant to retain the genotype of those which would be considered most desirable after appropriate field tests had been made.

Seed was harvested on an individual plant basis. Sufficient seed for field testing was obtained from 5 of the 6 plants in the first group and 28 of the 46 plants in the second group. Seed from these 33 individual plants, composite seed lots from each

[1] Plant Breeder and Chief Agronomist respectively, Holly Sugar Corporation, Sheridan, Wyoming.

of the two groups, and a few hybrid combinations were planted in the Imperial Valley on the Nelson Corral ranch in September 1958. The test was of rectangular lattice design with six replications. Plot size was one row by 50 feet.

Good stands were obtained for most entries and the plants were vigorous during the growing season. The beets were harvested in June, 1959.

Experimental Results

It is readily apparent that the differences measured in this test are significant at the 1% level.

Table 1.—Calculated F Values and 1% Level F Values for Characters Measured.

	Tons Beets	Percent Sucrose	Percent Purity
Calc. F Value	3.47	6.56	3.28
1% Level F Value	1.68	1.68	1.68

Table 2 gives the yield data of the 33 plant lines from the two groups.

Considering percent sucrose, every selected line is significantly greater than HC 1. It is also apparent that all five plants in the first group are generally higher in percent sucrose than the 28 plants in the second group. All the lines are better than HC 1 for percent purity, over half of them significantly so. Again, the five plants in the first group will average higher than the 28 plants in the second group. Most of the lines do not differ from the check in tonnage. Several of the lines are significantly above the check in sugar per acre.

A comparison of the composited seed lots and the parental material is shown in Table 3.

The composited seed of the five individual plant lines in the first group is 8231-0 and 8232-0 is the composited seed of the 28 individual plant lines in the second group. HH 3 is one of our best hybrids in the area and HC 1 is the parental material from which the selection was made.

A substantial increase in sucrose was shown by both composite seed lots, this with an increase in tonnage in the second group and a decrease in the first group, although these differences are not significant. This sucrose increase is reflected in a large sugar per acre increase, about 25 percent in the case of the second group and 12 percent in the case of the first group. An increase in purity of nearly two points for the second group and nearly three points for the first group is indicated.

Table 2.—Individual Plant Performance of Lines Tested When Ranked by Sugar per Acre.

Line	Sugar/Acre Lbs.	Beets/Acre Tons	Sucrose %	Purity %	Beets/100 Ft. Row
2-19	11055	40.056	13.80	88.4	145
2-31	11024	41.103	13.41	89.2	128
1-1	10764	37.928	14.19	89.2	136
2-9	10582	38.120	13.88	88.9	135
2-21	10578	38.353	13.79	89.9	142
1-4	10268	35.803	14.34	90.2	125
2-30	10248	37.347	13.72	89.0	131
2-10	10245	36.538	14.02	88.4	134
2-6	10230	39.559	12.93	88.0	127
2-32	10171	38.908	13.07	88.7	108
1-5	10059	35.772	14.06	89.2	136
2-20	9953	38.223	13.02	87.0	120
2-13	9920	38.419	12.91	88.1	104
2-2	9717	33.738	14.40	90.2	135
2-29	9635	34.984	13.77	88.7	138
2-27	9464	35.183	13.45	88.4	138
2-5	9332	34.616	13.48	87.8	140
1-2	9283	32.803	14.15	90.4	85
2-25	9116	35.117	12.98	88.8	107
2-24	9049	33.689	13.43	89.0	130
2-22	9046	32.632	13.86	90.4	120
2-7	9026	35.201	12.82	87.0	128
2-3	9014	33.584	13.42	90.2	110
2-26	9003	32.737	13.75	88.2	130
2-8	8910	32.952	13.52	88.4	112
1-6	8787	30.638	14.34	90.8	97
2-14	8700	31.704	13.72	89.2	124
2-23	8347	29.166	14.31	89.6	84
2-15	8343	31.842	13.10	89.4	114
HC 1	8203	34.122	12.02	86.8	137
2-18	8088	29.221	13.84	87.6	110
2-4	7695	28.865	13.33	90.0	103
2-11	7211	26.414	13.65	88.9	88
2-28	6871	26.247	13.09	88.3	90
LSD (5%)	1517	5.3	.70	2.0	

Table 3.—Production Data for Four Entries in the Imperial Valley 1958 Field Test.

Variety	Sugar/Acre Lbs.	Beets/Acre Tons	Sucrose %	Purity %	Beets/100 Ft. Row
8232-0	10247	38.466	13.32	88.6	128
8231-0	9266	32.696	14.17	89.6	112
HH 3	9225	35.729	12.91	89.0	144
HC 1	8203	34.122	12.02	86.8	137
LSD (5%)	1517	5.3	.72	2.0	

From these results it is readily apparent that the selection pressure for sucrose and purity was highly effective by use of the "Unit Block" selection method. Both sucrose and purity were increased substantially without decrease in tonnage as is usual when heavy selection pressure is applied for these characters. In the first group where the selection pressure for sucrose and purity was the greatest, the increase in sucrose and purity was the greatest. This was accomplished by some reduction in tonnage although the difference is not significant. In the second group the selection pressure was not as great for sucrose and purity and the increases were not as great. An increase in tonnage was obtained in this group but here again, the difference is not significant. However, it may be that since a larger number of roots were used in the second group, a wider base was maintained and the increase in tonnage over that of the first group may be a real difference.

Summary and Conclusions

Fifty-three roots were selected for sucrose and purity on a "Unit Block" basis in the Imperial Valley in California.

A progeny test of 33 of these roots which had produced seed in two group plantings showed substantial increases in sucrose and good increases in purity for all the lines tested.

Composite seed lots from these two group plantings showed substantial increases in sucrose, purity and sugar per acre.

It would seem that an increase of the composite 8232-0 would produce a variety superior to the parent HC 1 from which it was selected.

Further seed increases to be made utilizing the cuttings obtained from plants shown to be superior in the test should show additional improvement in sucrose, purity and sugar per acre.

Changes in the Concentration of Amino Acids in Sugar Beet Plants Induced by Virus Yellows

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Introduction

Investigations have shown that changes in the concentration of certain amino acids take place in the leaves of susceptible sugar beet plants infected with a virulent strain of the curly top virus (2)[2]. The studies reported here were concerned with the changes in the concentration of certain amino acids in the leaves of sugar beet plants infected with a virulent strain of beet yellows virus.

Methods

In the first test, 20 plants of sugar beet variety US 75, growing in 6-inch pots in the greenhouse, were selected for uniformity. Ten plants were inoculated in the 6 to 8-leaf stage with a severe strain of the yellows virus. The remaining plants served as controls. The plants were fertilized bi-weekly with Hoagland's solution containing 210 p.p.m. of nitrogen until they reached the 12 to 14-leaf stage, at which time leaf samples were taken.

The plants were watered late in the afternoon to make certain they were not under stress when leaf samples were taken the following morning. Leaf samples, representing three ages of leaves, were taken from each plant as follows: (a) The two youngest mature leaves, showing the typical chronic symptoms of thickening and yellowing, were taken from each of the diseased plants for individual plant analysis. Leaves of the same age were taken from the healthy plants for the controls. These leaves were not senescent but were designated as "old leaves" for contrast. (b) Two leaves which had just reached full size were taken from each of the diseased and healthy plants and designated as "mature leaves." (c) Two leaves which were approximately one-half fully expanded were also taken from the diseased and healthy plants and designated as "young leaves." The midribs were removed and the juice expressed from the quick-frozen blades at a pressure of 5000 pounds. The juice was preserved by the addition of a thymol crystal and a small amount of phenyl mercuric nitrate and stored at 10° F. The samples were thawed, shaken thoroughly, and centrifuged before aliquots were removed for analysis.

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[2] Numbers in parentheses refer to literature cited.

A second test was conducted to determine whether similar changes in the concentration of the same amino acids occur in yellows-affected leaves of sugar beets grown in the field. In this test, the amino acids were determined in only the mature, healthy and diseased, leaves taken from 123 breeders selections showing a range of reaction to yellows. One ¾-inch disk was removed from each of 10 mature leaves taken from different healthy plants of the same breeders selection and composited. Leaves of comparable age, showing the chronic symptoms of virus yellows, were sampled in a similar manner. The inoculated plants were growing in a row adjacent to the healthy plants.

The amino acids were identified by two-dimensional paper chromatography. The technique was a modification of the methods given by Block, Le Strange, and Zweig (1) and by Goodban, Stark, and Owens (3). For quantitative work one-dimensional papergrams were run by the ascending method using Whatman No. 3MM paper. The solvents were normal butanol-acetic acid-water (4:1:5), phenol saturated with water, and 2, 6-lutidine-ethanol-water (2:2:1) to which was added 12.5 ml. of diethylamine per liter of solvent. The concentration of cystine, alanine, tyrosine, r-aminobutyric acid, phenylalinine, and "the leucines" was determined using the butanol solvent, and valine and methionine were determined using the lutidine solvent. The concentration of the remaining amino acids was determined using water-saturated phenol.

Each leaf sample was spotted in triplicate with two healthy and 2 corresponding diseased samples on each sheet of paper. Two-microliter spots were used for the amino acids determined in phenol as the solvent, and 3 and 5 microliter spots were used for the amino acids run in the butanol and lutidine solvents, respectively. Five and 10 microliter spots were required to identify certain of the amino acids on 2-dimensional papergrams. The solvents were allowed to ascend the paper 18 to 19 cm. which required approximately 7½ hours at 25° C.

A standard 200 mg. % solution was prepared for each amino acid and dilutions made to give a series of concentrations of 100, 50, 25, 12.5, and 6.25 mg. %. Four 2-microliter spots of each dilution were placed on papers and run concomitantly in the same cabinet with the leaf samples.

The papers were dried overnight in a 10 cu. ft. oven holding a tray of water and maintained at 45° C. The papers were dipped in 0.3% ninhydrin in redistilled ethanol. The color was allowed to develop for a period of 4½ hours in an incubator maintained at 45° C. The relative humidity was maintained at approximately

50 percent with a moisture tray and a stream of preconditioned air passing through the incubator at a rate sufficient to replace the air three times during the color-developing period. The background color of the papergrams was very light and uniform, the transmission density being only slightly greater than that of the untreated paper.

The relative density of the spots of the standards was determined by a photovolt transmission densitometer unit using an aperture 4 mm. in diameter. The mean transmission density of the 4 replicated spots, for each of the concentrations, was plotted against the log of the concentration of the amino acid and a standard curve drawn. A densitometer reading, of the area having the maximum density, of each leaf-sample spot was made and the concentration of the amino acid taken from its standard curve.

Table 1.—The Concentration of Certain Amino Acids in the Expressed Juice of Leaves of Three Ages of Individual Healthy and Yellows-Affected Sugar Beet Plants Grown in the Greenhouse.[1]

	Concentration of Amino Acids (Milligram Percent)					
	Old Leaves		Mature Leaves		Young Leaves	
Amino Acid	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Aspartic Acid	28.9	16.4	32.8	27.6	27.4	18.5
Glutamic Acid	46.5	32.9	48.5	32.2	36.2	27.9
Citrulline	25.7	83.8	27.0	50.0	31.3	32.6
"Leucines"	9.7	25.7	4.7	11.2	9.5	12.3
Threonine	4.7	21.1	3.6	6.6	5.8	5.2
β-Alanine	19.7	30.0	12.4	17.0	22.3	21.0
γ-aminobutyric	11.8	15.2	12.4	19.0	17.5	18.3
Valine	12.1	21.7	10.0	15.0	8.1	9.8
Methionine	13.4	18.3	9.0	10.5	7.7	8.0
Tyrosine	16.1	17.0	14.0	13.1	36.2	37.9
Alanine	6.1	6.0	6.0	7.1	10.1	10.2
Cystine	6.3	4.4	7.1	7.2	10.3	9.3
Serine	2.7	4.1	4.4	5.4	6.6	6.4
Glycine	2.3	2.2	4.1	7.6	.....	.....

[1] Mean values for 10 plants.

Results

Table 1 shows the mean concentration of the amino acids determined in three ages of leaves from 10 healthy and 10 diseased plants grown in the greenhouse. Aspartic acid and glutamic acid were the only amino acids determined which showed

a decrease in concentration in the diseased leaves. The decrease occurred in all ages of leaves but not to the same extent. In the old and mature leaves, citrulline, the "leucines," threonine, p-alanine, r-aminobutyric, alanine, valine, and methionine increased in concentration, whereas the concentration of serine, tyrosine, cystine, and glycine remained unchanged. The "leucines" were the only amino acids to show an increase in concentration in all ages of leaves. The young leaves appeared to be affected least by the disease. Except for the decrease in the concentration of aspartic and glutamic acids and the increase in the "leucines," the concentrations of the remaining amino acids were unchanged. Arginine, histidine, and lysine were also found to be present, the highest concentration being found in the old leaves. Of the three, arginine appeared to be present in highest concentration. In some samples the concentration reached 3 mg. percent.

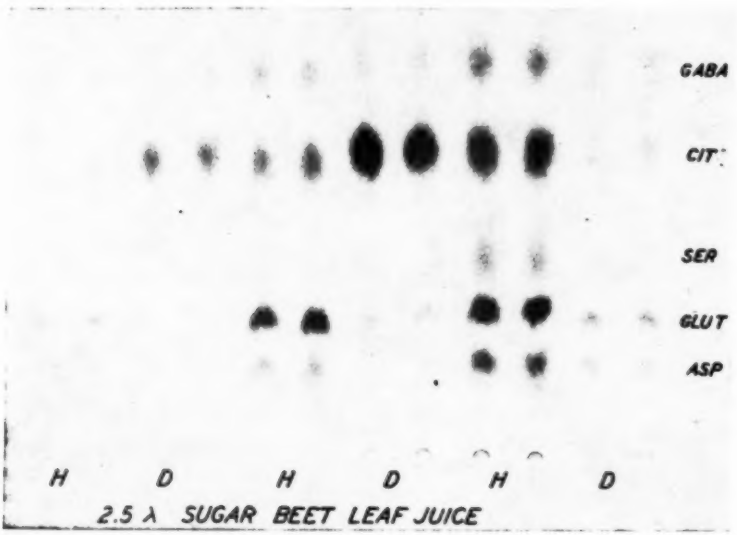

Figure 1.—Papergram, developed in water-saturated phenol, of juice expressed from mature leaves of 3 healthy plants and juice from leaves of comparable age from 3 plants having yellows showing. Aspartic acid (asp.), glutamic acid (glut), serine (ser), citrulline (cit), and -aminobutyric acid (gaba). Other amino acids are present with r-aminobutyric acid. H-Healthy leaf, D-Diseased leaf.

Figure 1 is a typical papergram, developed in water-saturated phenol, showing spots of certain amino acids in the juice expressed from healthy mature beet leaves and from juice expressed from leaves of similar age showing the chronic symptoms of yellows.

A relationship which may be useful in diagnosing the disease in individual plants is a ratio of the amino acids which decrease to those that increase in the leaves as a result of the disease. For example, if the ratio $\frac{\text{aspartic acid plus glutamic acid}}{\text{citrulline plus alanine}}$ (alinine calculated as citrulline) is calculated for the leaves of healthy and diseased plants, values are obtained which are different. The amino acid ratios are shown in Table 2 for three ages of leaves from 10 healthy and 10 diseased plants grown in the greenhouse. The greatest difference in the amino acid ratio between the healthy and diseased plants was obtained with the leaves old enough to show chronic symptoms of the disease.

Table 2.—The Amino Acid Ratio[1] in the Expressed Juice from Leaves of Three Ages from Healthy and Yellows-Affected Sugar Beet Plants Grown in the Greenhouse.

Plant	Old Leaves		Mature Leaves		Young Leaves	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
1	3.03	0.67	2.63	2.66	2.00	1.12
2	2.00	0.34	2.59	0.94	1.12	0.75
3	1.58	0.76	2.14	0.56	0.96	0.62
4	2.52	0.35	3.70	0.88	1.02	0.87
5	1.75	0.36	2.42	0.67	1.67	1.29
6	2.42	0.44	2.86	1.06	1.94	1.29
7	1.89	0.59	1.65	1.10	1.56	0.79
8	1.90	0.42	1.14	0.63	0.88	0.95
9	2.34	0.37	1.80	1.23	1.63	1.09
10	1.17	0.35	1.19	0.79	1.31	1.06
Mean	2.06	0.46	2.21	1.05	1.41	0.98

[1] $\frac{\text{Aspartic Acid + Glutamic Acid}}{\text{Citrulline + Alanine}}$

In this test, the mean ratio for the old leaves of the healthy plants was approximately 4.5 times greater than the ratio for the old leaves of diseased plants. The ratios did not overlap.

The wide variation in the amino acid ratio of the leaves showing the chronic symptoms indicated that all plants were not affected by the disease to the same extent. In view of this vari-

ation in the amino acid ratio among individual plants, it appears that it may be possible to select plants by an amino acid ratio that may have a higher degree of resistance to virus yellows.

In the second test, which was conducted on field-grown plants, a highly significant reduction in the concentration of aspartic acid and glutamic acid also occurred in the leaves of the diseased plants. The mean reduction in concentration for these amino acids was 47 and 40 percent, respectively. The concentration of citrulline and alanine increased 37 percent in the same diseased plants over that of the healthy plants.

The amino acid ratio separated the healthy and diseased plants into two groups. The mean amino acid ratio for the healthy plants was 1.41 as compared with 0.62 for the diseased plants. Based on analysis of leaves of healthy plants, only 9 of the 123 selections had ratios which would classify them as diseased plants. Conversely, in an analysis of leaves of diseased plants, only 8 of the 123 selections had ratios which fell in the range covered by the healthy plants.

Of the 123 breeders selections, four were of the type which remain relatively green even though the plants are stunted by the disease. These selections are designated by some breeders as "green selections." The mean amino acid ratio for these "green selections" was 1.60 and 0.57 for the healthy and inoculated plants respectively. It appears, therefore, that the changes in the concentration of the amino acids, as a result of the disease, may not be directly correlated with the yellowing of the infected plants.

Summary

The concentration of certain amino acids was determined, by paper chromatography, in the juice expressed from three different ages of leaves taken from healthy and from yellows-infected beet plants grown in the greenhouse and from mature leaves taken from sugar beet selections grown in the field. The concentration of aspartic acid and glutamic acid decreased in all ages of leaves, whereas all the other amino acids determined either increased or remained unchanged. The greatest changes in the concentration of the amino acids occurred in the old leaves that showed the typical chronic symptoms of the disease. In the young leaves, aside from a reduction in the concentration of aspartic and glutamic acids, only the "leucines" increased. The concentrations of the remaining amino acids were unchanged. Differences in the concentration of certain amino acids in the leaves of healthy and yellow-affected sugar beet plants were found

to be sufficiently high and constant to suggest that a ratio of the amino acids involved may be useful as an aid in diagnosis.

A considerable variation in the concentration of certain amino acids occurred among sugar beet plants affected with yellows. This indicates that it may be possible to select individual plants which may have resistance to sugar beet yellows on the basis of the changes in the concentration of certain amino acids in the mature leaves showing the chronic symptoms of the disease.

Literature Cited

(1) Block, R. J., Le Strange, R., and Zweig, G. 1952. "Paper chromatography," pp. 51-60, New York, Academic Press, Inc.

(2) Fife, J. M. 1956. Changes in the concentration of amino acids in the leaves of sugar beet plants affected with curly top. Jour. Amer. Soc. Sugar Beet Tech. IX (3): 207-211.

(3) Goodban, A. E., Stark, J. B., and Owens, H. S. 1953. Amino acids-content of sugar beet processing juices. Jour. Agr. and Food Chemistry. 1 (3): 261-264.

Processing Monogerm Seed

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Monogerm seed will have an advantage over processed multigerm seed only if its viable germs can be more uniformly distributed in the row. Two monogerm seeds per cell in the seed drill can result in as closely a paired condition on emergence as would be obtained with double germed processed seed. It is therefore obvious that the processing and grading of monogerm seed must be improved to produce a degree of uniformity in size and shape that will permit precision planting. At the same time the diameter and thickness of the drill cell must closely match the diameter and thickness of such carefully processed and graded seed. There is also the additional practical necessity at this stage of eliminating contaminating multigerm from monogerm seed.

Monogerm seed has a different shape than multigerm seed. This is well illustrated in Figure 1, drawn by Mrs. Merle Pierpoint of the Agricultural Experiment Station, Corvallis, Oregon. As shown, monogerm seed is disc like in shape, while multigerm clusters tend to be round in general outline.

The Blackwelder decorticator, in general use for processing multigerm seed, rolls the seed between a stone and a rubber disc. In doing so it breaks up the larger seed clusters, removes part of the cork, and the resulting particles tend to be spherical in general shape. This machine does not work quite so well with flat monogerm seed since fracturing of a seed cluster is obviously not required. The soft cork to be removed is attached only on the edges of the flat monogerm seed while it is attached irregularly on all surfaces of the multigerm seed. It was therefore concluded that a processing machine of different design might give better results.

The accepted practice of using round-holed screens to grade multigerm seed has its disadvantages for use with flat monogerm seed. Such screens measure in one dimension only and do not eliminate seeds which are too thin or too long. The inclusion of these abnormally shaped seeds requires the use of larger drill cell openings which in turn increase the chances of getting two seeds per cell of a drill plate which defeats the purpose of monogerm seed. This situation has forced certain processors to issue as many as four sizes of seed by grading between screens differing by only one-sixty fourth of an inch in round hole dimensions.

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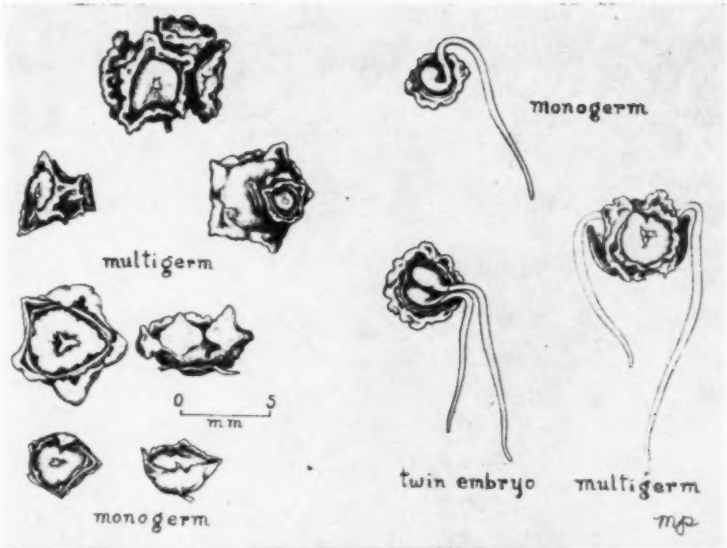

Figure 1.—A comparison of monogerm and multigerm seed size and shapes (drawings by Mrs. Merle Pierpont, Agricultural Experiment Station, Corvallis, Oregon).

Polishing and Two-Dimensional Grading

A special monogerm polisher was designed, built, and put into operation in Ladner, British Columbia, in conjunction with a two-dimensional grading system.

The first step in the process is to remove only a minimum amount of the soft cork surrounding the seed so that it will pass through a 10/64 round hole. This insures that the seed is below 10/64 in one dimension. This operation is accomplished by the machine illustrated in Figure 2. Here the seed is tumbled in a cylinder made of a screen with 10/64 round holes. Inside the cylinder are a set of discs with abrasive surfaces revolving in the opposite direction at about 800 R.P.M. When sufficient cork is removed the seed drops through the round openings. Since the seed is tumbling when ground, only the corky edges are worn down with no damage to the flat surface.

The dust and small seed is subsequently removed by passing the seed over a 7/64 round hole screen. The seed is then passed over a No. 13 indent cylinder which removes all of the seeds that are longer than 10/64 in any dimension. This is in essence sorting the seed for acceptance in a 10/64 drill plate opening.

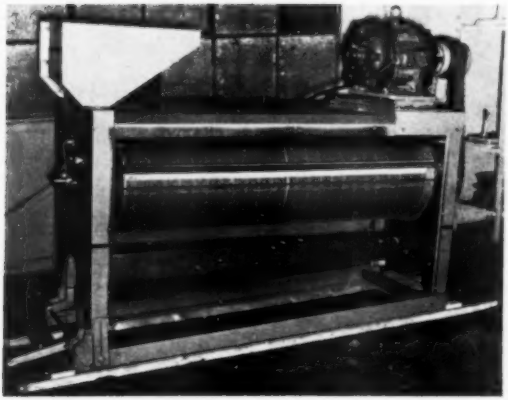

Figure 2.—A special monogerm polisher which removes a minimum amount of soft seed cork to permit passage through a 10/64 round-hole screen.

The rejected particles are recycled for further polishing while the selected seed is then ready for final grading. The monogerm fraction of the seed is a flat disc less than 10/64 in the largest dimension, while its other dimension varies from 5/64 to 10/64. Preliminary examinations of seed which tended to be close to 10/64 in both dimensions, or round in general shape, showed that this seed usually contained two or more germs, while the flatter seeds were always monogerm.

A wide selection of ribbed and slotted grading cylinders was made available for testing by the Hart Emerson Simon Company in Winnipeg. The ribs on these screens are designed to tip all disc-like objects on edge so that a genuine grading for thickness is achieved. The results of this grading are summarized in Table 1. It can be seen that seed passing through a 6¼/64 slot was 100% monogerm, while seed passing over a 7/64 was 91% multigerm.

The thickness range selected to suit the degree of precision required for planting under Alberta conditions was 1¾/64, and consisted of seed passing over a 5¼/64 ribbed slot and through a 7/64 ribbed slot. Seed thinner than 5¼/64 was discarded as it was found to have only 55% germination, while seed larger than 7/64 was round in shape and mainly multigerm in nature and was to be processed and sold as multigerm seed. The selected final sample (Table 2) was 94% monogerm and was used in the drill tests reported below.

Table 1.—Thickness Grading with Ribbed Slotted Precision Cylinders.

Description	Width of Slot	Percent of Ungraded Sample	Percent Monogerm	Percent Germination	Weight (grams) per 100 Germs
Ungraded	.....	100	71	76	
Through	5/64	1.8	100	53	
Through	5¼/64	2.1	100	65	
Through	5½/64	6.7	100	61	.279
Through	5¾/64	13.6	100	64	
Through	6/64	7.6	100	64	
Through	6¼/64	13.4	100	69	
Through	6½/64	7.5	95	71	
Through	7/64	16.2	85	73	.277
Through	7½/64	14.5	41	83	
Through	8/64	11.1	6	82	.273
Over	8/64	5.5	3	91	

Table 2.—Commercial Thickness Grading.

Description	Width of Slot	Percent of Ungraded Sample	Percent Monogerm	Percent Germination
Ungraded	.....	100	71	76
Through (Test II)	5¼/64	4	100	55
Through (Test II)	7/64	61	94	72
Over (Test II)	7/64	35	22	83
Through (Commercial)	5¼/64	8	100	
Through (Commercial)	7/64	67	92	
Over (Commercial)	7/64	25	6	

Pollution of monogerm with multigerm can arise from several sources. There is a planned pollution when a multigerm pollinator is used with a male-sterile monogerm. About 7% of the blend planted is multigerm pollinator but it usually produces more than 7% of the plants at harvest time. There is also the possibility of pollution by volunteer multigerms, with the further possibility that the original stock seed was not genetically pure monogerm. In all of these situations the need to separate multigerm from monogerm is obvious. Now that they can be separated it may be advantageous in many cases to use a multigerm pollinator with assurance that it can be cheaply removed from the hybrid seed. This would be of particular value where the yielding ability of the pollinator was greatly inferior to the hybrid as is usually the case, particularly if the pollinator is an inbred strain.

Germination of Monogerm Seed

The minimum germination for beet seed under the Canada Seeds Act is 75%. This was easily achieved under normal conditions. However, such will not be the case with monogerm seed as seen in Tables 1 and 2. Here the highest germination of pure monogerm was 69% while multigerm grown in the same field under identical growing and processing conditions germinated up to 91%. On a mathematical probability prediction basis where the germinability of single germs was 75% then the germination of multigerm seed would be 96%. These calculations were based on multigerm seed having 50% doubles, 30% triples, and 20% quadruples. Our experience to date, therefore, indicates that the standards of germination in monogerm may have to be modified in line with practical experience.

Germ Size

The weight per 100 germs was established for three thickness grades as shown in Table 1 by excising and weighing the germs. It was somewhat surprising to find the germ weight similar in all the samples. We expected to find that the monogerm germs would be heavier than the multigerm germs. While there was a slight trend in this direction it was likely too small to be of significance. There is no explanation as to why the 5½/64 seed should have as heavy a germ as the 7/64 seed.

Monogerm Drill Tests

Extensive laboratory drill tests were run by the Agricultural Department of Canadian Sugar Factories in Alberta, using drill cell dimensions to match closely the diameter and thickness of the monogerm seed processed by the new equipment. I.H.C. and John Deere drill units were mounted over a belt conveyor so that the moving belt approximated ground travel speed and drove the seed drill mechanism. The seed was dropped on to greased boards and its distribution was determined. Operating the units on the belt without the grease board for the equivalent of 1/100 acre drill travel permitted the seed and grindings to be collected and weighed. In these tests the 1960 thickness-graded seed was compared with the 8-10/64 round screen graded seed used in 1959. Although the trials included two ground speeds of 1.9 and 2.7 m.p.h. as well as spacings of 1, 1.5, and 2 inches, only the data on the faster speed of 2.7 m.p.h. and the widest spacing of 2 inches are reported in Table 3. Inclusion of the additional data would not alter the conclusions.

Table 3.—Monogerm Drill Tests.

Drill speed 2.7 M.P.H.

Spacing JD = 2.1 I.A.C. = 2.0 inches
Seed/Ft. JD = 5.7 I.A.C. = 6.0 inches

Type of Seed	Drill	Seed Plate Cell Size	% Grindings	Seed Distribution 100" Grease Board								
				Singles	Doubles	Multi.	Actual No. Seeds	Est. No. Seeds	Ratio A/E	Seeds per Foot	Actual Lbs./A.	Est. Lbs./A.
1959 8-10	JD	10x8	3.84	39.5	10	0	59.5	47.6	1.25	7.14	3.10	2.48
1959 8-10	JD	10x7	3.47	44.5	2	0	48.5	47.6	1.02	5.82	2.52	2.48
1959 8-10	JD	10x6	2.80	41.5	3	0	47.5	47.6	1.00	5.70	2.47	2.48
1960 5¼-7/-10	JD	10x8	3.20	40.5	4	0.5	50.0	47.6	1.05	6.00	2.90	2.76
1960 5¼-7/-10	JD	10x7	4.09	41.5	3	0	47.5	47.6	1.00	5.70	2.76	2.76
1960 5¼-7/-10	JD	10x6	3.47	44.5	1.5	0	47.5	47.6	1.00	5.70	2.76	2.76
1959 8-10	IHC	10x8	5.95	41.5	11	2.5	71.0	50.0	1.42	8.52	3.70	2.60
1959 8-10	IHC	10x7	4.82	35.5	9	0.5	55.0	50.0	1.10	6.60	2.87	2.60
1959 8-10	IHC	10x6	2.52	39.5	6	0	51.5	50.0	1.03	6.18	2.68	2.60
1959 8-10	JD	10.7x8	4.87	44.5	6	0.5	57.0	47.6	1.20	6.84	2.97	2.48
1959 8-10	JD	10.7x6	2.65	43.5	2.5	0.5	49.0	47.6	1.03	5.88	2.55	2.48
1960 5¼-7/-10	JD	10.7x8	3.62	44.5	4	0	52.5	47.6	1.10	6.30	3.05	2.76
1960 5¼-7/-10	JD	10.7x7	3.14	39.5	4	0	47.5	47.6	1.00	5.70	2.76	2.76
1960 5¼-7/-10	JD	10.7x6	4.34	40.5	2	0	44.5	47.6	0.93	5.34	2.58	2.76
1960 5¼-7/-10	IHC	11x8	6.74	41.0	10	0.5	62.5	50.0	1.25	7.50	3.63	2.90
1960 5¼-7/-10	IHC	11x7	1.40	43.5	6.5	1.0	59.5	50.0	1.19	7.14	3.45	2.90
1960 5¼-7/-10	IHC	11x6	2.20	38.5	6.5	0	51.5	50.0	1.03	6.18	2.99	2.90
1960 5¼-7/-10	JD	11.2x8	2.59	46.0	8.5	1	66.0	47.6	1.38	7.92	3.83	2 76
1960 5¼-7/-10	JD	11.2x7	2.09	48.0	4	0	56.0	47.6	1.17	6.72	3.25	2.76
1960 5¼-7/-10	JD	11.2x6	2.38	38.5	3	0.5	46.0	47.6	0.96	5.52	2.67	2.76

Plate thickness—In all of the comparisons, 8/64 was found to be too thick and permitted the sowing of too many doubles. When plates with 10/64 diameter holes were used, a plate thickness of 6/64 or 7/64 gave almost perfect distribution in most cases with the actual number of seeds and pounds per acre matching the estimated amounts very closely. Where cell diameters of 10.7, 11, and 11.2/64 were used the thinnest plates gave the best results in all but one instance.

Cell diameter—The preferred cell diameter is 10/64 with acceptable results from the 10.7/64 dimension. Diameter dimensions appear to be less critical than thickness dimensions. It is suspected that the use of the indent cylinder to remove off-shaped seeds has permitted the close matching of the drill size with the maximum seed diameter.

Type of drill—The results did not indicate any preference as regards to make of drill. Both gave equally satisfactory results when the proper plate thickness and cell diameter were used.

Grading procedures—The processing procedure followed in 1960 is recommended over that used in 1959, as the thickness grading of seed eliminated the thin seeds below 5¼/64, and the round multigerm above 7/64. It also permitted the inclusion in the same commercial seed of plump germs with a diameter below 8/64 which had to be discarded in the 1959 seed.

Conclusions

1. A method of monogerm seed processing and grading has been developed which has the following characteristics:
   a. Removes unnecessary cork without any apparent damage to the germ.
   b. Maintains the diameter of the seed as close to 10/64 as possible.
   c. Eliminates off-shaped particles through the use of indent cylinders.
   d. Eliminates thin germs by the use of ribbed cylinders with slots 5¼/64 wide.
   e. Eliminates a high proportion of the multigerms by the use of ribbed cylinders with slots 7/64 wide. The elimination of round particles permits the use of thin plates to match the disc shape of the monogerm.
2. Drill tests indicate the suitability for precision planting of drill plates 6/64 to 7/64 thick with cells 10/64 in diameter.
3. The practice of subdividing monogerm seeds into two or more size classes through the use of standard screens now appears unnecessary.

Ion Exchange Process for Beet Sugar Refining
I. Purification of Beet Diffusion Juice

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Before describing the technique known as the Assalini "A" Process, it is advisable to summarize briefly the conventional method for extracting sugar from beets as practiced in most of Europe. The procedure is outlined in Figure 1. European practice differs chiefly in that a raw beet sugar is produced. This sugar is remelted and passed through some decolorizing material and finally crystallized to obtain a refined sugar meeting the specific chemical and physical requirements of the particular country. As in the U.S., the greater demand in the European market is for refined sugar.

In the past 10 or 15 years, ion exchange resins have aroused considerable interest in the sugar industry in general, inasmuch as this technique showed considerable promise as a new method

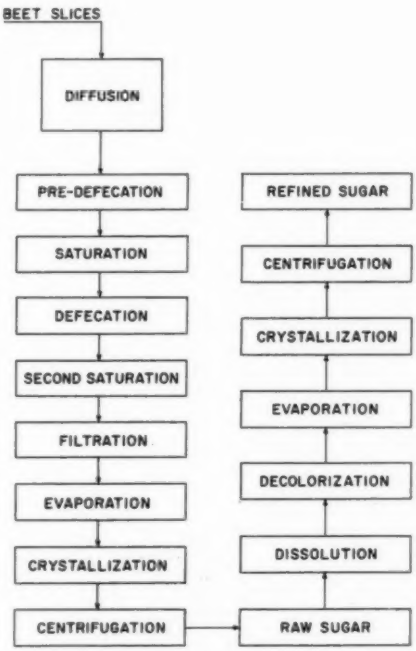

Figure 1.—European beet sugar process.

of purification which could be capable of augmenting crystallization as the purifying technique. As a first approach, the application of ion exchange resins was investigated either for the purification of thin juices to obtain greater sugar recovery or to purify the molasses to recover the sugar which is otherwise uncrystallizable.

In large measure, the application of ion exchange resins for deionization has not yet yielded the outstanding results originally hoped for. The reasons for this have been many and the difficulties might be summarized as follows:

Thin Juice Deionization

When using conventional deionization for the purification of thin juice, it is necessary to cool the juice from 90° C. down to 10°-15° C. when using a strongly acidic cation exchange resin. This is necessary, of course, to avoid serious inversion of the sucrose during and immediately after the passage of the juice through the bed of cation resin in the hydrogen form. Inversion can be controlled by use of a carboxylic cation exchanger. Additionally, when using strongly basic quaternary ammonium anion exchangers, it is necessary to cool the juice to 60° C. or below to avoid undesirably high degradation of the functional groups. While heat losses may be kept to a minimum, a heat-exchange installation adds significantly to the capital investment.

Needless to say, all such expenses both for new plant and processing costs must be borne entirely by the additional sucrose extracted beyond that for the conventional method and must also cover the value of the molasses no longer produced.

Molasses Purification

Ion exchange installations for purification of molasses would not require special cooling since these syrups could be treated during the off-season. The major problem encountered in the purification of molasses is that of dilution. In fact, for the deionization of molasses, it is generally necessary to make successive dilutions. First, the viscosity of the molasses must be reduced by dilution from the point of view of lowering the concentration of non-sugars so that the ion exchange operation is more easily controlled. Second, the sugar must be rinsed from the columns after exhaustion which entails additional dilution. Because of the high non-sugar content, the cycles are relatively short and the sweetening-on and sweetening-off portion of the cycle becomes a large percentage of the throughput.

In an attempt to simplify the conventional sugar recovery process along with increasing the quantity and quality of sugar

produced, it was decided to study the purification of diffusion juice directly by means of ion exchange with the elimination of all of the stages of defecation in the conventional process. Toward this end, a series of investigations were performed, initially in small columns on synthetic juice and later in a pilot plant capable of processing 180 gallons of diffusion juice per cycle. A schematic flow sheet of this plant is shown in Figure 2.

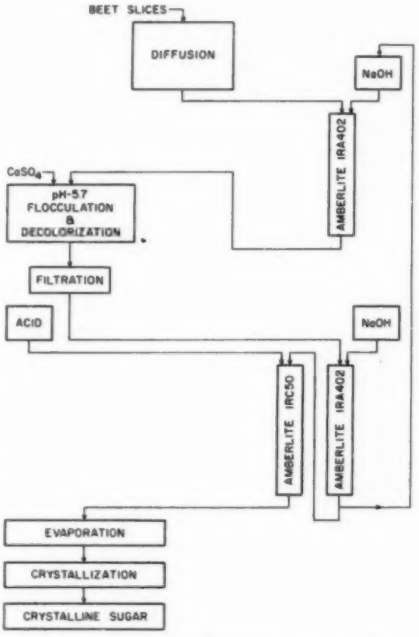

Figure 2.—Application of Assalini "A" Process to European beet sugar production.

The scheme of this process could be summarized as follows:

First Stage

This consists of passing the diffusion juice, as it comes from the measuring tanks, through a bed of strongly basic anion exchange resin principally in the hydroxide form. This resin should be of the porous variety such as Amberlite IRA-401S or, preferably Amberlite IRA-402. The mineral salts present are converted as in normal ion exchange with the adsorption of the anion and the conversion of the cation to its corresponding

hydroxide. The complex organic salts are partly dissociated by the exchange reaction which results in an increase in pH due to the conversion of the mineral cation to its hydroxide. The pH of the diffusion juice, originally between 5.5 and 6.5, reaches a value around pH 10 after passage through this resin column thereby eliminating the immediate possibility of serious inversion.

Second Stage

(Chemical treatment and filtration)—The sugar juice, which has already been partly defecated by the ion exchange treatment, is subjected to a chemical treatment with an inorganic salt which is able to form a "floc," and thus further defecate and decolorize the juice. This particular phase of the process required a very large number of experiments in order to determine the most suitable salts for the floc formation which was optimum from the point of view of analytical, technical, and economic requirements. It was found that a most efficient flocculation of the organic substances present, along with nearly complete decolorization, could be obtained using a 30% solution of aluminum sulfate. This treatment must be effected with special care since a pH of 5.7 must be obtained to form the aluminum hydroxide floc. This pH value is optimum from the point of view of best defecation, easiest filtration and minimum dissolution of aluminum sulfate into the juice which would, of course, increase the ash content. Even under these conditions, however, filtration is rather difficult and commercially practical filtration rates can be obtained only with vacuum filters. This treatment is also more expensive than those which will be described below but it is interesting to observe that, at a relatively low pH for a sugar juice, no increase in invert content was observed even at rather high temperatures.

The use of calcium chloride for this flocculation was also investigated. This has given very good results from the point of view of analyses and economy. In this case, the juice from the first ion exchange column is treated with a 30% solution of calcium chloride. With the beet juice under investigation, it was found that optimum defecation was reached when a quantity of calcium chloride equal to about 0.03% of juice was employed. Flocculation was excellent as was the decolorization. Moreover, the filtration of the suspension was easier than that resulting from the aluminum sulfate treatment. Naturally, the juice defecated in this way undergoes a further increase in purity. However, it is necessary to avoid using excessive amounts of calcium chloride because of the obvious increase in ash content which would result from this very soluble salt. Economically,

the use of calcium chloride was considerably more desirable than the aluminum sulfate treatment because of the small quantities employed.

It was found that calcium sulfate is the salt which yields the most interesting results from the industrial point of view especially from the viewpoint of lowest cost. The amount of anhydrous calcium sulfate which proved to be optimum on the juice investigated was 0.3 to 0.4% based on juice. Addition of the calcium sulfate was done from a slurry of calcium sulfate. The addition was made to a sugar liquor having a pH of 9.9. The final pH was adjusted to 7.7 for ease of handling. The flocculation was almost complete and the decantation of the precipitate was very rapid. This would permit the use of thickeners already present in the factory and the filtration of the thickened slurry presented no special difficulties.

Ordinary sand filters have been employed for these filtrations since they were found entirely suitable. It should be noted that, in the case of the calcium salts, the juice does not reach an acidic pH since the hydroxides formed after passage through the anionic resin in the first stage remain either substantially unchanged or are only partly converted to the corresponding sulfate.

Third Stage

After the filtration indicated above, the juice still contains amounts of soluble minerals and organic substances. In order to complete the purification and decolorization, the juice is sent through two further beds of ion exchange resins. The first is a porous anion exchange resin in the hydroxide form, preferably Amberlite IRA-402, and the second is a weakly acidic cation exchanger in the hydrogen form, preferably Amberlite IRC-50. After this two-bed deionization, the juice has a very high purity, is virtually colorless and yields sugars equivalent in quality to the products achieved by the refining process used in Europe as described previously.

During this final ion exchange treatment, the pH never falls below 7 but it is possible to obtain a final juice with a higher pH value by reducing the level of regeneration of the Amberlite IRC-50. However, this was found to be unnecessary during the entire investigation. Since the carboxylic cation resin is weakly acidic, its regenerant consumption for sulfuric acid is only slightly higher than theoretical. While the use of a strongly acidic cation resin might give a higher purity with the removal of betaine, this substitution would naturally give rise to significant inversion.

The anion exchanger in the first stage is regenerated with the excess caustic recovered from the regeneration of the anion exchanger in the third stage. This technique is important, firstly, from the point of view of economy since it permits a high regeneration level of the anion exchanger in the deionization stage, which gives maximum purity, while the excess hydroxide ion can be utilized in the first stage anion exchanger to bring about the necessary pH rise. Secondly, the regenerant effluent from the second anion exchanger contains a large quantity of various sodium salts in addition to the excess sodium hydroxide which serves as the actual regenerant for the first anion exchanger. These sodium salts serve to remove the great bulk of the organic material adsorbed by the first anion exchanger which treats the diffusion juice directly.

The anion exchanger in the first stage need not be regenerated to high levels, since even if all of the salts in the diffusion juice are not converted to the corresponding hydroxide, there is always a good increase in purity and the pH rises to a point such that flocculation, either with aluminum or calcium sulfate, is easily brought about and is quite efficient.

As regards the analytical and economic data obtained from the various experiments, the following summaries indicate the experimental results obtained using the process described above. Incidentally, the analytical data used for the calculation of technical and economic balances, have been obtained enzymatically as regards the sucrose and by a solids balance as regards the non-sugars. The yields and extractabilities were obtained by means of direct weights. These results were in agreement with calculations made according to the usual extractability formula.

Table 1.—Analytical Data on Assalini "A" Process.

	Diffusion Juice	Final Juice "A" Process
Enzymatic sucrose—%	15.50	15.40
True dry substance—%	18.23	15.80
Enzymatic true purity—%	85.0	97.5
Total non-sugars—%	2.73	0.40
Ash—%	0.61	trace
Chlorides—%	0.043	trace
Invert—%	0.033	0.036
Color (Stammer units—100% solids)	360	0.097
pH	5.80	7.70

The juice obtained with the application of the "A" Process, on evaporation and crystallization, produces a thick juice and a first product massecuite with the same characteristics but with a

slight increase in the purity due to the removal of volatile non-sugars during evaporation. This resulted in a slight decrease in pH due to the volatilization of nitrogen compounds. A comparison of yields between the Assalini "A" Process and conventional European practice is given in Table 2.

Table 2.—Operation Results.

	Conventional European Practice	Assalini "A"
Sucrose—%	16	16
Losses up to raw juice—%	0.50	0.50
Lime process losses—%	0.10	—
Resin and filtration process losses—%	—	0.10
Sucrose in juice—%	15.40	15.40
Purity—%	90	97.5
Total non-sugar—%	1.71	0.40
Sucrose in molasses (purity 60)	2.57	0.60
Extractible sucrose	12.83	14.80
Extractible gained 100 lbs. beets	—	1.97

Table 3.—Operating Costs-Assalini "A" Process (Based on Chemical Prices in Italy).

	Cents/100 lbs. Beets
Reagents	
Sulfuric acid (66 Be) 0.44 lbs.	0.580
100% caustic soda 0.70 lbs.	2.860
Calcium sulfate 0.300 lbs.	0.071
	3.511
Other Expenses	
Steam 7 lbs.	0.819
Labor for ion exchange—0.01 hour	0.293
Labor for filtration 0.01 hour	0.293
	1.405
TOTAL	4.92 cents

Table 4.—Economics of "A" Process.

Raw and Crystalline Sugar	
Abolition—Lime Process	2.20 cents/100 lbs. beets
Cost "A" Process	4.92 cents/100 lbs. beets
Net Cost "A" Process	2.72 cents/100 lbs. beets
Processing Cost per Pound Sugar Gained	1.38 cents/lb. sugar
Value of Sugar in Molasses not Produced	2.31 cents/lb. sugar
Cost of Sugar Gained	3.69 cents/lb. sugar

Economics

Raw and Refined Sugar Production

The economics of the "A" Process have been evaluated in Table 4. Here again the values are based on Italian sugar practice.

Production of Refined Sugar

From a syrup with a purity of 97.5 to 98% and 0.5 Stammer units per 100 solids, at least 50% of refined sugar is obtained in Europe directly from the first massecuite. The second and third products would be sent back to the refinery. Taking the cost of refining at 0.73¢/lb., we have:

Table 5.—Production—Refined Sugar.

Expense with the conventional process 0.73 cents × 12.83 =		9.4 cents
Assalini "A" Process expense	$\frac{0.73 \times 14.80}{2} =$	5.4 cents
Saving for 100 lbs. beets		4.0 cents
Saving for each lb. of additional sugar:	$\frac{4.0 - 2.72}{1.97} =$	0.64 cents/lb.

The cost for a plant to process 2000 tons of beets per day has been estimated in Table 6. The economic evaluation has again been based on European practice.

Table 6.—Plant Economics—"A" Process.

Basis—2,000 tons beets per day
Installation: $210,000—Resins
$113,000—Equipment

$323,000—TOTAL

Amortization Rate—5 campaigns—300 days

	Crystalline	Refined
Destroyed Molasses	2.310	2.310
Amortization	1.242	1.242
Operating Expenses	1.386	.....
TOTAL	4.938	3.552
Lowered Cost	.....	0.637
Total Cost—1 lb. additional sugar—produced, cents	4.938	2.915

At this date, extensive testing has not been completed to give an accurate field estimate of exchanger life but the above value is believed to be realistic.

Ion Exchange Process for Beet Sugar Refining
II. Purification of Sugar Solutions

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From an economic point of view, it would be of interest to the beet sugar industry to purify, between campaigns, by-products which are stable to storage. The exploitation of ion exchange installations to be used for this purpose, can be spread over 12 months instead of two or three months as for installations which treat beet juice only during the campaign.

A process has been studied which employs ion exchange resins for the purification of molasses inexpensively. During the sugar campaign, this process is able to treat, instead of molasses, the mother liquor of 75% purity which comes from the centrifugation of the first product which, in the European system, roughly resembles low-machine syrup in the U. S. beet industry. The mother liquor so treated has the same purity and density characteristics as a defecated thin juice before evaporation. Thus, during the campaign, the sugar factory could produce only first product raw sugar, eliminating most of the concentration, centrifugation, and conditioning stages of the low-grade products.

The so-called Assalini "B" Process involves only a partial removal of mineral salts and organic non-sugars from molasses by means of a rather novel ion exchange technique. A brief description of the operational method is as follows:

The molasses from the conventional process is diluted with an equal volume of certain fractions of the effluents from the "B" Process which are called "recycles." These recycles have a Brix of 10 or higher and a sugar content of 5 to 6%. This dilution is necessary both to decrease the viscosity of the molasses and facilitate its passage through the resin bed and to obtain a concentration of non-sugars sufficiently low to facilitate their separation from the sucrose in the ion exchange process. All of the solutions are at room temperature.

The installation consists of two columns and the flow pattern is shown in Figure 1. The first contains a porous anion exchanger such as Amberlite IRA-401S and the other a cationic resin such as Amberlite XE-200, a strongly acidic, porous cation exchanger in the 30-50 mesh particle size range. Both columns contain the same quantity of resin. Taking the resin volume of each column as 100, the following solutions are charged:

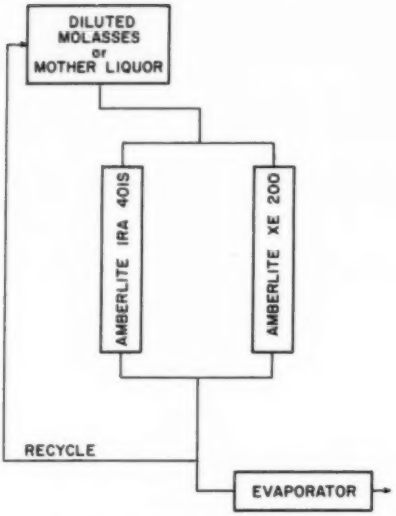

Figure 1.—Assalini "B" process.

Amberlite IRA-401S Influents

1. 20% resin volume of diluted molasses
2. 100% resin volume of water to displace the sucrose from the resin
3. 12% resin volume of 5% sodium hydroxide solution
4. 48% resin volume of water to wash the caustic from the resin

In all, for each cycle, there is charged a total volume equal to 180% of the resin volume.

Amberlite XE-200 Influents

1. 20% resin volume of the diluted molasses
2. 80% resin volume of water to displace sucrose from the resin
3. 12% resin volume of 10% sulfuric acid solution
4. 68% resin volume of water to wash the acid from the resin

Again, the charge of liquid equals 180% of the resin volume.

The effluents are taken off in different fractions as follows:

Table 1.—Amberlite IRA-401S Effluents.

1st Fraction:	67% resin volume contains non-sugars and a little sucrose.
2nd Fraction:	10% resin volume which represents the recycle which is mixed with the cationic column recycle for the dilution of molasses for the next cycle.
3rd Fraction:	70% resin volume. This contains the purified sucrose liquor which, after mixing with the purified effluent from the cationic resin, goes to evaporation.
4th Fraction:	33% resin volume. This contains non-sugars, spent regenerants and a little sucrose.

The average chemical analyses of these effluents are shown in Figure 2.

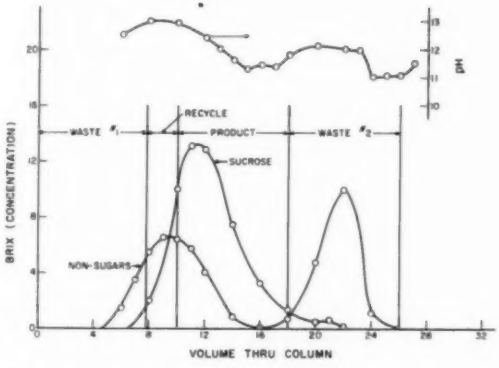

Figure 2.—Typical effluent pattern for anion exchange bed, Assalini "B" Process.

Table 2.—Amberlite XE-200 Effluents.

1st Fraction:	40% resin volume containing a little non-sugar and traces of sucrose.
2nd Fraction:	44% resin volume representing the purified portion. This is mixed with Fraction 3 of the anionic effluent and is evaporated to produce refined sugar.
3rd Fraction:	10% resin volume. This is the recycle which, when mixed with Fraction 2 of the anionic column, is used for the dilution of the molasses.
4th Fraction:	86% resin volume containing non-sugars, spent regenerant, and a little sucrose.

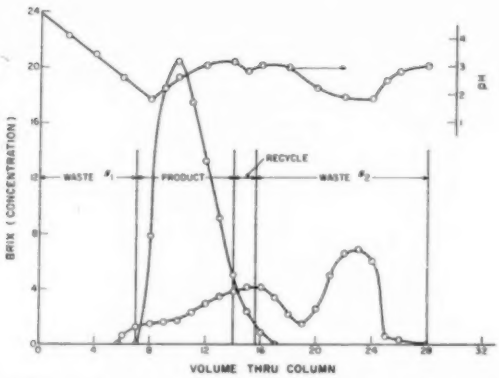

Figure 3.—Typical effluent pattern for cation exchange bed, Assalini "B" process. Product fraction: upper curve—sucrose; lower curve—non-sugars.

The average chemical analysis of these effluents are shown in Figure 3.

Fractions No. 1 and 4 from both the anion and cation columns are sent to waste since they contain the displaced water, little sucrose and a great deal of the non-sugars present in the molasses. Additionally, they contain the various spent reagents which have been introduced into the columns such as caustic and sulfuric acid.

The average composition of these liquors is summarized in Tables 3 and 4. These values are reported on a solids bases.

Table 3 gives the over-all effect of the "B" process on the final product.

Table 3.—Average Analysis of Assalini "B" Process Liquors.

Component	Original Molasses	Thick Syrup	Evaporated Massecuite	Raw Sugar	Residual Molasses
Sucrose-polarimeter corrected	51.00	47.14	73.52	89.57	53.13
Sucrose—true	52.24	45.91	72.66	87.77	51.44
Percentage solids, refractivity	82.11	59.88	92.79	97.47	86.41
Percentage solids, true	81.57	60.12	93.15	97.83	86.71
Purity ratio, refractivity	62.11	78.72	76.66	91.89	61.48
Purity ratio, true	64.04	76.36	78.00	89.72	59.32
pH	10.30	9.38	9.17	9.10	9.50
Invert, % true sucrose	0.019	0.414	0.276	0.051	0.916
Total nitrogen, % true sucrose	3.38	.....	1.47	0.44	3.72
Total non-sugars, true value	29.33	14.21	20.49	10.06	35.27
Mineral non-sugars	11.94	.....	6.82	2.81	11.77
Organic non-sugars	17.39	.....	13.67	7.25	23.70
Organic non-sugars from nitrogen	11.03	.....	6.69	2.44	11.97
Organic non-sugars, non-nitrogen	6.36		6.98	4.81	11.53

Table 4.—Average Analysis of Assalini "B" Process Liquors.

Component	First Anion Fraction	First Cation Fraction	Fourth Anion Fraction	Fourth Cation Fraction
Sucrose—polarimeter	0.33	0.52	1.29	0.35
Solids—refractivity	4.55	1.60	3.00	5.00
pH	12.0	2.80	12.0	1.90
Invert, % true solids	trace	trace	trace	0.37
% nitrogen, % true solids	3.18	4.06	2.80	3.48
Total non-sugars	4.22	1.08	1.71	4.65
Mineral non-sugars	1.77	0.20	0.70	2.12
Organic non-sugars	2.45	0.88	1.01	2.53
Organic non-sugars, nitrogen	0.91	0.41	0.52	1.09
Organic non-sugars, non-nitrogen	1.54	0.47	0.49	1.44

Fractions No. 3 and 2 respectively of the anion and cation resins when mixed together, represent the product portion which is sent to evaporation. Its average composition is as follows:

Brix	12.5-13.5
Polarization	10 -10.5
Purity	78 -80
pH	8 -10

The color (Stammer units—100% solids) is lower than that of the normal juice of the same purity.

The syrup obtained on evaporation of the product fractions corresponds approximately to the syrup which is obtained after centrifugation of the first product in European beet factories. Thus, the sugar which can be obtained is a normal second product sugar.

The mother liquor from the centrifugation of the massecuite is in turn a molasses which has the same characteristics as the ordinary final molasses in Europe. This is true since the organic and inorganic non-sugars are removed in approximately the same ratio as they occur in the original molasses. Consequently, the "secondary" molasses obtained from the "B" Process contains roughly the same ratio of impurities as the original molasses treated. It should be noted that this secondary molasses has a refractometric purity higher than the true purity which is the reverse of that usually observed for the molasses obtained in the conventional way.

The analytical data of the recycles are not included in the table since they are used to dilute the molasses for subsequent cycles.

The exact mechanism of the Assalini "B" Process is not known. However, it is apparent that the extremely low level of regenerations used, roughly one-sixth of that which would be normally employed in conventional deionization, results in a relatively small stratum of regenerated resin in the upper part of the column. It is possible that, as the regenerant ions are displaced from this portion of the column, successive ion exchange processes occur farther down in the bed. Ion exclusion plays a role in this process. Of interest to the mechanism of the process is is the fact that the void volume of the exchangers is approximately 40 percent.

The application of the "B" Process gives interesting economic results under European conditions where the Steffen Process is almost never used. Using a molasses with a purity of 60 and considering this as a limiting value for further crystallization, a purity of about 80 can be obtained in the purified liquor. In this case, it is possible to extract 50% of the sucrose originally present in the molasses. When processing mother liquor coming from the centrifugation of the first factory product, which has a purity of about 75, juices are obtained with a purity of about 90. This would represent about 28% more extractable sucrose as compared to that extractable with the conventional method without application of the "B" Process.

The operating expenses necessary to achieve these levels of purification are quite low. The materials consumed are as follows:

Sulfuric acid (66 Be):	0.099 lbs./lb. of sucrose in molasses treated
Sodium hydroxide 100%:	0.05 lbs./lb. of sucrose in molasses treated
Fuel Oil:	0.274 lbs./lb. of sucrose in molasses treated
Water:	3 U.S. gallons/lb. of sucrose in molasses treated

These consumptions are obviously very small and represent an economical method for extracting sugar from low-grade products.

The optimum conditions of operation using a typical molasses from an Italian beet factory, are indicated in the following table:

	Anionic Column	Cationic Column
pH	5 to 10	5 to 10
Maximum temperature	—	20° to 25° C.
Resin bed height	160 cms. (5.25 ft.)	160 cms. (5.25 ft.)
Time per cycle, minutes	120′	120′
Operating flow rate	0.015 liters/liter resin/min. (0.11 gpm/ft^3)	0.015 liters/liter resin/min. (0.11 gpm/ft^3)

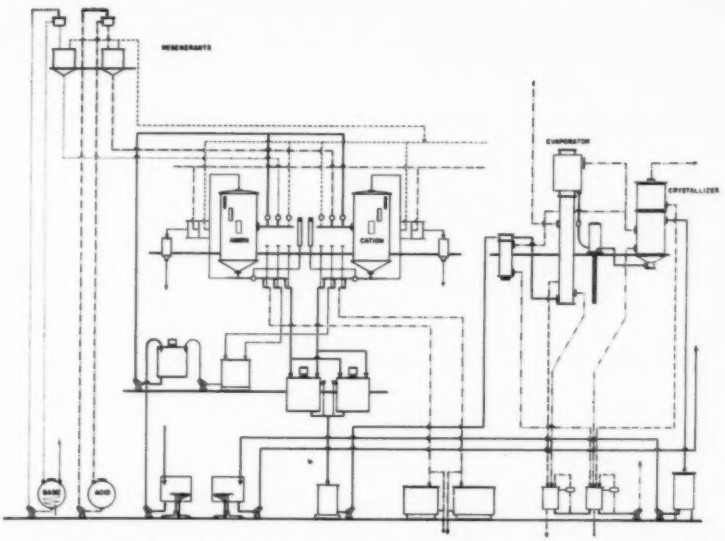

Figure 4.—Flow diagram for an industrial plant. Heavy line indicates sucrose piping.

Table 5.—Income from Assalini "B" Process.

Sucrose in molasses charged	15,300 tons
% extractable—54.61	
Total extractable sugar	8,355 tons
$\frac{8355}{0.7867}$	10,621 tons raw sugar to be refined
Refinery losses 0.80% of 10,621 ton = 85 tons	10,536 tons (after losses)
The extractable is 10,536 tons × 0.7867	8,288 tons of 100% saccharose
$\frac{8288}{0.998}$	8,305 tons refined sugar
$\frac{8305}{0.9912}$	8,379 tons refined sugar-gross weight
8,379-tons at $205.00/ton	$1,717,695.00
15,300 × 0.4039 = 6180 tons 6180 tons × $50.80	$ 314.000.00
Total income—sucrose + sucrose in molasses	$2.031,695.00
Refining Costs	
8,379 tons at $11.30/ton	$ 94,500.00

Table 6.—Cost and Income for an Assalini "B" Installation with a Capacity of 30,000 Tons of Molasses per Year.

	Cost	Income
30,000 tons molasses 51% sucrose in molasses (15,300 tons) at $50.80/ton	$ 778,000.00	
Cost for processing with resins		
1,680,000 of 100% NaOH at 4.1 cents/lb. (0.05 lb./lb. of sucrose in molasses or 1.96 lb. molasses)	69,100.00	
3,400,000 of H_2SO_4 at 1.24 cents/lb. (0.099 lb./lb. sucrose in molasses or of 1.96 lb. molasses)	42,300.00	
Labor—0.02 cents/lb. of sucrose in molasses	6,120.00	
Operating cost—other than resins		
Labor—0.0785 cents/lb. of sucrose in molasses	24,050.00	
Fuel Oil (three-effect evaporation) 0.274 lb./lb. of sucrose in molasses 8,400,000 lbs. of naphtha at 1.09 cents/lb.	91,500.00	
Cost of refining	94,500.00	
Interest on resin plant (10% per year on $96,500)	9,650.00	
Amortization of resin plant of 20% per year	19,300.00	
Value of the refined sugar in bags		$1,717,695.00
Value of sucrose in by-product molasses		314,000.00
TOTAL COST	$1,134,520.00	
TOTAL INCOME		$2,031,695.00
NET INCOME	$ 897,175.00	
	$2,031,695.00	$2,031,695.00

Figure 4 shows a schematic diagram of an industrial plant.

The economic balance is developed in Tables 5 and 6. Here the cost figures refer to Italian practice. The results indicate the advantages to be obtained with the "B" Process in Italy. The extractability of sugar has been based on the equation:

$$\% \text{ theoretically recovered} = \frac{100\ (X - 0.6)}{X\ (1.0 - 0.6)}$$

where X is the purity of the treated sugar effluent. The value 0.6 stems from the purity of molasses which is the end by-product of the sugar refining operation. No indication of the number of crystallization steps can be given. The figure 0.7867 refers to the purity of the effluent liquor. The value 0.546 was obtained

from the above equation using a purity of 78.67%. The figure 0.9980 refers to the moisture content of commecial sugar in Italy. In Italy the weight of the sugar is sold as gross weight in bags so that the factor 0.9912 is used to correct for this value. Commercial value for sugar and molasses refer to average sugar prices in Italy at the time the paper was written.

The cost of refining sugar from the "B" Process liquor is different ($11.30/ton) than for the cost stated in Part I for an "A" Process liquor ($14.20/ton). This is due to the fact that the "B" liquor does not require the coagulation and filtration steps required in the conventional process.

Since this paper was presented, additional work has been reported on these processes. These papers and patents are listed below (1-4). An accurate life estimate for the resin has not been obtained as yet. Pilot plant work indicates no definite trend in operating characteristics for the resin but the amount of cycles still is not sufficient to project the thousands to be expected under life operation.

1. Schiwek, H. 1959. Zeitsch. Zuckerind. 84: 502-508.
2. Schiwek, H. 1960. Zeitsch. Zuckerind. 85: 342-349.
3. Assalini, G. U. S. Patent 2,929,745: Mar. 22, 1960.
4. Assalini, G. U. S. Patent 2,929,746: Mar. 22, 1960.

Northern California Sugar Beet Quality Survey

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Many fields of sugar beets being harvested in Holly Sugar Corporation's Northern California districts in 1958 were considerably lower in quality than in previous years. The percent sugar and percent purity of beets in many fields had decreased to a critically low point. During the fall of 1958 and again in the summer and fall of 1959 extensive field sampling was conducted throughout the Northern California District to determine, if possible, some of the factors responsible for this low quality. Fields were sampled throughout the district to obtain beet and petiole samples for analysis. Information was compiled regarding disease and insect conditions, cultural practices, growth characteristics, and many other factors that would contribute to the study of the fields. Company fieldmen and their growers cooperated with the workers conducting the survey and contributed the desired field information.

This paper will describe the procedures used in the survey, the general disease and insect observations compiled, and a summary of the initial data derived from the program in 1958 and 1959. Results are based on data from field samples taken at or near harvest time.

Procedure

Holly's Northern California District was divided into four areas to be surveyed: South San Joaquin, Tracy-Stockton, Sacramento and Hamilton City. In 1958 sampling began on September 10 and was completed by November 13. In the 1959 program, sampling began on July 8 and ended December 2. The main difference between the two years was that fewer fields were sampled in 1959, however, each field was sampled at about three week intervals until harvest. Only the results of the last sampling date will appear in this report. The following numbers of fields were sampled in each area:

	1958	1959
South San Joaquin area	66	60
Tracy-Stockton area	48	31
Sacramento area	55	31
Hamilton City area	60	30
Total Northern California	229	152

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From each field in the survey, four beet root samples (10 feet of row per sample) were dug and sent to the laboratory for sugar (polarity) and purity (apparent) determinations. Petioles were obtained from the beets in each sample and were later analyzed for nitrate-nitrogen content by the Holly Sugar Research Department Laboratory. Growth characteristics, soil moisture conditions, insect and disease damage, and type of planting were recorded at the time of sampling.

During the period of the survey, fieldmen also obtained information regarding fertilizer usage, crop history and other items pertinent to each field sampled. Data were computed in a manner to furnish an average figure for petiole nitrate-nitrogen (p.p.m), sugar percent, purity percent, weight per beet and calculated yield (tons/acre) for each field. The initial data derived from the survey for both 1958 and 1959 are presented and summarized.

Observations

In 1958 two diseases were of major importance in this district. In the Hamilton City area *Cercospora* leafspot was extensive in all fields sampled and harvested early in the season. In over half of the fields in the South San Joaquin area leafspot symptoms were present. Virus yellows occurred in the South San Joaquin and Tracy-Stockton areas with light to heavy infestations.

Empoasca leafhopper damage was believed to be of a moderate nature in all of the areas in the survey. A few fields contained noticeable root aphid populations and/or nematode damage. Other disease and insect damage was slight.

Disease and insect damage in the fields included in the 1959 survey was somewhat less than in 1958 and probably had little effect on production.

Results

From the 1958 field samplings, petiole nitrate-nitrogen (p.p.m.), sugar content, purity, average weight of beets, units of nitrogen applied, and the number of days between the last application of nitrogen, and the sampling date were recorded for each field. Following this analysis, the fields were divided on the basis of the amount of nitrate-nitrogen contained in the petioles at harvest. For both years the fields were separated into ones high in No_3-N and low in No_3-N. Table 1 is a summary of the results of analysis of samples from the two categories in the Northern California district. All figures in the table are averages for the number of fields sampled. In 1958, the fields in the low nitrate-nitrogen (less than 500 p.p.m.) category, with an average of 337 p.p.m., were higher in percent sugar and purity

Table 1.—Summary of Field Sampling and Survey of Sugar Beet Fields in the Holly Sugar Corporation in Northern California, 1958 and 1959.

NO_3-N Level	No. Fields	NO_3-N	Sugar	Purity	Beet Avg. Wt.	Tons/A.	Units N/Acre	Days-Last N to Sampling
		p.p.m.	%	%	lbs.		lbs.	
1958								
Low	76	337	15.54	85.06	1.54	23.9	126[1]	133[1]
High	153	2337	13.25	80.32	1.67	23.7	139[2]	126[2]
Difference		2000	2.29	4.74	.13	.2	13	7
1959								
Low	61	842	15.01	82.22	1.66	28.40	128[3]	51[3]
High	91	3090	13.84	80.18	1.76	28.42	124[4]	54[4]
Difference		2248	1.17	2.04	.10	.02	4	3

[1] Average of 65 fields
[2] Average of 145 fields
[3] Average of 51 fields
[4] Average of 69 fields

than the fields in the high nitrate-nitrogen category with an average of 2337 p.p.m. The low nitrate-nitrogen fields averaged 2.29% higher sugar and 4.74% higher in purity. Difference in average weight per beet was .13 pound in favor of the high nitrate-nitrogen fields. Yield was nearly the same for the two categories with only .2 ton per acre difference. Even though nitrate usage and days between application and sampling were similar for the low and high categories, the high nitrate-nitrogen levels appeared to be at least partially responsible for the differences obtained in the sugar and purity percentages.

In 1959 the data were secured on a similar basis and compiled for direct comparisons. The separation point for nitrate-nitrogen was somewhat higher than the 500 p.p.m. used in 1958. The low NO_3-N class included approximately two-fifths of the fields, and the high NO_3-N class approximately three-fifths of the fields. Fields averaging as high as 1200 p.p.m. NO_3-N were included in the low category.

Table 1 also shows the low and high averages determined by petiole nitrate-nitrogen level for all the fields studied in 1959. Percent sugar and percent purity in the low NO_3-N class were both higher than at the high NO_3-N level. The percent sugar averaged 15.01% for the 61 fields with a 842 p.p.m. average NO_3-N level. However, the 91 fields averaging 3090 p.p.m. NO_3-N tested only 13.84% sugar or 1.17% less than the fields

in the low nitrogen category. Purity percentages were similar in that the fields in the high nitrogen category averaged 2.04% less in purity than in the low nitrogen category. Beets in the high nitrogen fields weighed .10 pound heavier than the beets from low nitrogen fields but this was not considered significant. Differences between calculated yield (tons/acre), units of N, and days between last N application and sampling were also quite small. Although the difference in sugar percent between the two categories (low and high NO_3-N) was lower in 1959 than in 1958 it was concluded that nitrogen in the petioles at harvest time was the main factor responsible for this difference.

Summary

Although units of nitrogen applied and the days between nitrogen application and sampling were very close between the high and low categories in both 1958 and 1959, low sugar content and purity was always associated with high nitrate-nitrogen in the petioles. The average yield (tons/acre) for both 1958 and 1959 was not significantly affected by the difference in the nitrate-nitrogen level.

It was found that in 1958 two diseases, *Cercospora* leafspot and virus yellows, were of major importance in three of the areas in the Northern California District. Undoubtedly, these two diseases were responsible for some of the low quality beets harvested in 1958. In 1959 diseases were moderate in comparison to 1958 and were not considered as being responsible for any large reduction in quality. However, additional data in Table 1 has shown that excess nitrate-nitrogen in the plants at harvest time was partially responsible for the low quality beets harvested in both 1958 and 1959. In the areas surveyed, all fields in the low sugar-low purity category averaged considerably higher than 1,000 p.p.m. nitrate-nitrogen at harvest time. This would indicate that excess nitrogen was present and should be considered an important factor in causing low quality beets in our Northern California District.

Notes Section

Equipment for metering, distributing and mixing granular herbicides into bands. Weed control in sugar beets, in particular those weeds in the adjacent three to four inches of the row, has received considerable attention the last few years. Liquid herbicides applied in a water carrier have given good results under favorable conditions; however, granular type carriers may have better residual characteristics and promise to provide for easier handling by the farmer. Because of the potential advantages for granular carriers, the Agricultural Engineering Section of the University of Wyoming, in cooperation with The Great Western Sugar Company and the Holly Sugar Corporation, has been working on metering, distributing and mixing equipment for granular herbicides in connection with weed control in sugar beets. The work to date has resulted in the development of a machine for the band application of both spray and granular herbicides.

Experimental work on metering has resulted in the development of principles necessary for accurate metering; some of which are being used by manufacturers of equipment. Grinding due to excessive agitation was found to be one of the causes for inaccurate metering. The moisture content of the attaclay carrier, which changes with the relative humidity of the atmosphere affects the weight-metering rate. However, the error in calibration will be small if the attaclay is treated and the calibra-

Figure 1.—The machine development for the band application of granular and spray herbicides. The distributing device and spray nozzles are located ahead of four rotary-tillage units. A four-row planter is pulled by the tractor.

tion of the metering device is performed when the attaclay is in equilibrium with atmospheres between 30 and 75 percent relative humidity.

A band distributor consisting of a series of 1/8 and 7/16 inch pins was developed and found to give a uniform distribution pattern for a six-inch band.

The mixing of the granules into the upper one to two inches of soil was more uniform for the rotary-tillage and finger weeder units than for other mixing devices worked with.

A more complete report of the work appears in Vol. 3, No. 2 of the Transactions of the American Society of Agricultural Engineers. A copy of the report can be secured from the authors.

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Crop Science is the name of the new research journal which will appear in February 1961, as the official publication of the Crop Science Society of America, an affiliate of the American Society of Agronomy.

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